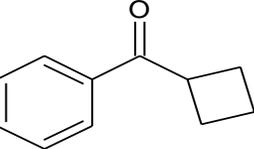
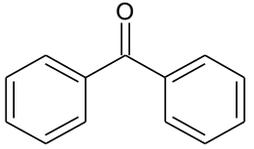
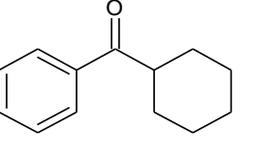
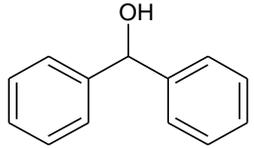
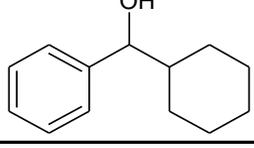


**Table 1. Standards available for identification and their characteristic physical and analytical parameters.**

| Chemical                              | Structure   | Molecular formula                 | CAS Number | MW g/mol | Boiling point °C | log K <sub>ow</sub> <sup>a</sup> | GC-MS              |                              |              | LC-MS        |                |                              |                        |
|---------------------------------------|---|-----------------------------------|------------|----------|------------------|----------------------------------|--------------------|------------------------------|--------------|--------------|----------------|------------------------------|------------------------|
|                                       |   |                                   |            |          |                  |                                  | t <sub>R</sub> min | r <sub>12</sub> <sup>b</sup> | Mol. ion m/z | Base ion m/z | Other ions m/z | r <sub>12</sub> <sup>b</sup> | [M+H] <sup>+</sup> m/z |
| <b>Parent</b>                         |   |                                   |            |          |                  |                                  |                    |                              |              |              |                |                              |                        |
| CBP: Cyclobutylphenyl ketone          |    | C <sub>11</sub> H <sub>12</sub> O | 5407-98-7  | 160.2    | 261              | 2.96                             | 11                 | 0.820                        | 160          | 105          | 77             | 0.765                        | 161.096                |
| DPK: Diphenyl ketone (Benzophenone)   |    | C <sub>13</sub> H <sub>10</sub> O | 119-61-9   | 182.2    | 305              | 3.15                             | 14                 | 1.020                        | 182          | 105          | 77/51          | 0.836                        | 183.080                |
| CPK: Cyclohexyl phenyl ketone         |    | C <sub>13</sub> H <sub>16</sub> O | 712-50-5   | 188.3    | 287              | 3.94                             | 14                 | 1.000                        | 188          | 105          | 77/133         | 1.000                        | 189.127                |
| <b>Metabolites</b>                    |   |                                   |            |          |                  |                                  |                    |                              |              |              |                |                              |                        |
| BADPK: Diphenyl methanol (Benzhydrol) |   | C <sub>13</sub> H <sub>12</sub> O | 91-01-0    | 184.2    | 312              | 2.71                             | 14                 | 1.041                        | 184          | 107          | 79             | 0.778                        | 185.096                |
| CPKOH:Cyclohexyl phenyl methanol      |  | C <sub>13</sub> H <sub>18</sub> O | 945-49-3   | 190.3    | 300              | 3.76                             | 14                 | 1.001                        | 190          | 107          | 79             | 0.975                        | 191.143                |

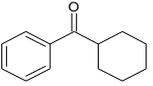
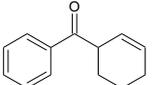
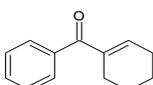
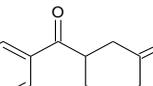
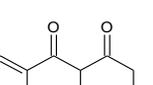
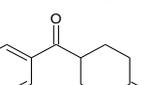
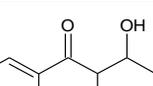
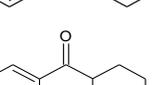
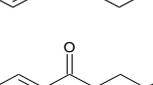
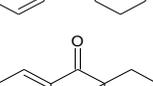
<sup>a</sup>log K<sub>ow</sub> denotes octanol/water partitioning coefficient obtained from Sci Finder (ACD Lab) or Suite v4.11(USEPA)

<sup>b</sup>r<sub>12</sub> denotes relative retention defined as a ratio of retention times t<sub>R</sub>/t<sub>RCPK</sub>

**Table 2. Experimental analytical conditions used for GC-MS and LC-MS analyses at USEPA and University of North Dakota (UND)**

| GC-MS system 1  | GC-MS system 2  | LC-MS/MS USEPA   | LC-TOF-MS UND   |
|---|---|--|---|
| <p>Column: HP-5MS, 5% phenyl–95% methyl-polysiloxane, 30 m, 0.25 mm I.D., 0.25 μm</p> <p>Carrier: He 1.2 mL/min</p> <p>Pulsed splitless injection 25 psi for 0.6 min, 1.0 μL; Tinjector 280°C</p> <p>Oven: 40 °C/1 min; 10 °C/min to 200 °C; 20 °C/min to 310 °C and 9 min hold.</p> <p>Ttransfer line = 280 °C</p> <p>MS: EI and CI; 50-500 <i>m/z</i></p> | <p>Column: DB5-MS, 5% phenyl–95% methyl-polysiloxane, 25 m, 0.25mm I.D., 0.25 μm</p> <p>Carrier: He 1.2 mL/min</p> <p>Pulsed splitless injection 25 psi, 0.45 min, 1.0 μL; Tinjector 250 °C</p> <p>Oven: 40 °C/1 min; 10 °C/min; 280 °C; 20 °C/min to 310 °C and 9 min hold.</p> <p>Ttransfer line = 280 °C</p> <p>MS: EI 50–500 <i>m/z</i></p> | <p>Column: Agilent SB-RP C18, 2.1 x 50 mm I.D., 1.8 μm</p> <p>Mobile phase A: 2% MeOH, 2% EtOH, 2.5 mM NH<sub>4</sub>OAc in water</p> <p>B: 2% EtOH, 96% MeOH, 2.5mM NH<sub>4</sub>OAc in water; 0-1.25 min 35% B; 13.5-18.0 min 65% B; 18.0-18.25 min 35% B</p> <p>Flow rate: 0.25 mL/min</p>   |   |
|   |   | <p>ESI: capillary, tube lens, cone, and extractor at 3100, 0.1, 65 and 3.8 V, respectively; capillary temperature 325 °C. N<sub>2</sub> sheath 30 psi, N<sub>2</sub> auxiliary 10 psi.</p> <p>CID at 30 eV (N<sub>2</sub>) with 0.1 scan for transition and mass range for products: 50-500 <i>m/z</i>. Transition dwell times: 8 and 4 and 2 ms for qualifier ion and internal Std, respectively.</p> | <p>ESI: capillary 4000 V; fragmentor 135 -250 V, 300 °C; N<sub>2</sub> nebulizing 25 psi, N<sub>2</sub> drying 12L/min; mass range: 50-500 <i>m/z</i></p> |

**Table 3. Identification of CPK metabolites based on GC-MS, chemical derivatization and LC-TOF-MS data**

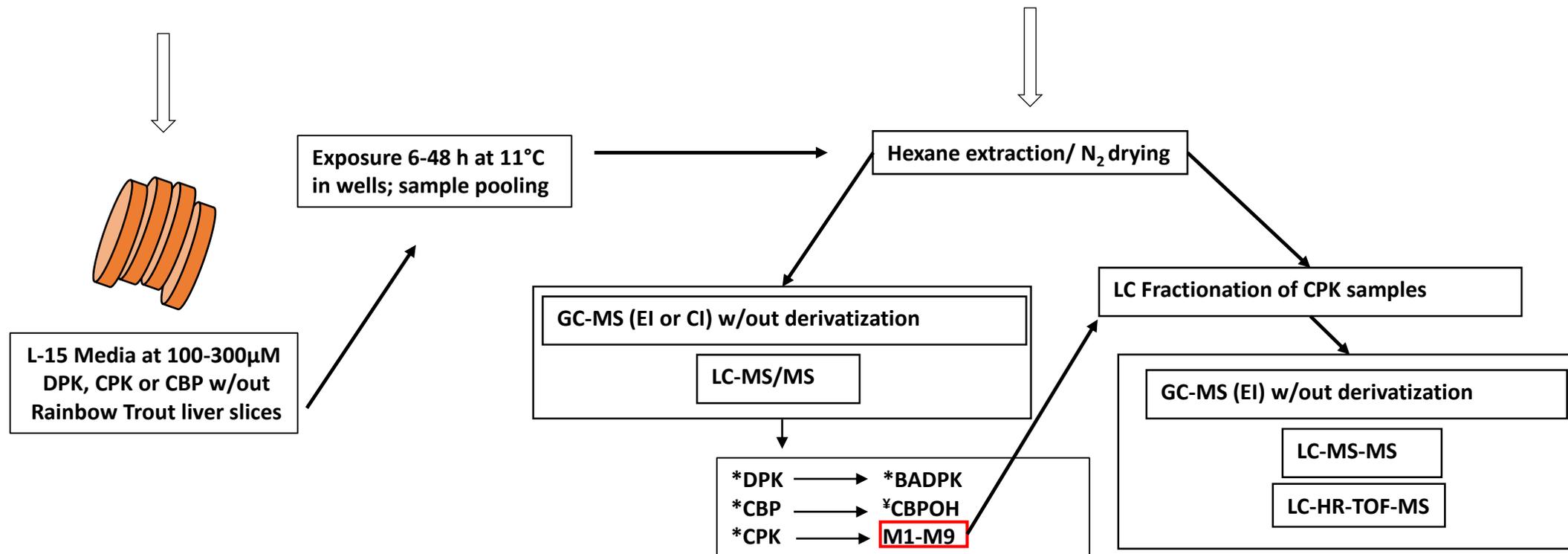
| Label | Structure   | Molecular formula                              | CAS Number   | Derivatives of phenyl ketones | MW g/mol  | *log K <sub>ow</sub> | GC-MS                |                                   |             |             | GC-MS & BSTFA        |          |   | LC-ToF-MS            |                        | mass error, ppm |          |      |
|-------|---|--|--------------|-------------------------------|-----------|----------------------|----------------------|-----------------------------------|-------------|-------------|----------------------|----------|---|----------------------|------------------------|-----------------|----------|------|
|       |   |  |              |                               |           |                      | t <sub>R</sub> , min | t <sub>R</sub> /t <sub>RCPK</sub> | El Ions m/z | Cl Ions m/z | t <sub>R</sub> , min | Mol. ion | Ions m/z                                | t <sub>R</sub> , min | [M+H] <sup>+</sup> m/z |                 |          |      |
| CPK   |    | C <sub>13</sub> H <sub>16</sub> O              | 712-50-5     | cyclohexyl-                   | 188.22    | 3.94                 | 15.68                | 1.000                             | 188/105/77  |             |                      |          | not derivatized                         | 19.3                 |                        |                 |          |      |
| M1    |    | C <sub>13</sub> H <sub>14</sub> O              | 29373-01-1   | cyclohexenyl-                 | 186.24966 | 3.02                 | ± 0.25               | 15.814                            | 1.009       | 186/105/55  | 187/105              |          | not derivatized                         | ND                   |                        |                 |          |      |
| M2    |    | C <sub>13</sub> H <sub>14</sub> O              | 17040-65-2   | cyclohexenyl-                 | 186.24966 | 3.382                | ± 0.29               | 16.343                            | 1.042       | 186/105/55  | 187/105              |          | not derivatized                         | ND                   |                        |                 |          |      |
| M3    |    | C <sub>13</sub> H <sub>14</sub> O <sub>2</sub> | 1046191-22-3 | cyclohexenone-                | 202.24906 | 1.78                 | ± 0.31               | 18.022                            | 1.149       | 202/105/77  | 203/187/105          |          | not derivatized                         | 4.78                 | 203.1069               | 1.2             |          |      |
| M4    |    | C <sub>13</sub> H <sub>14</sub> O <sub>2</sub> | 200624-56-2  | cyclohexenone-                | 202.24906 | 1.91                 | ± 0.31               | 18.062                            | 1.152       | 202/105/77  | 203/187/105          |          | not derivatized                         | 7.94                 | 203.1053               | -6.7            |          |      |
| M5    |    | C <sub>13</sub> H <sub>14</sub> O <sub>2</sub> | 908592-00-7  | cyclohexenone-                | 202.24906 | 1.997                | ± 0.32               | 18.161                            | 1.158       | 202/105/77  | 203/187/105          |          | not derivatized                         | 7.08                 | 203.1058               | -4.2            |          |      |
| M6    |    | C <sub>13</sub> H <sub>16</sub> O <sub>2</sub> | 1114462-94-0 | cyclohexanol-                 | 204.26    | 2.16                 | ± 0.24               | 18.119                            | 1.156       | 186/105/77  |                      |          | 16.858                                  | 276                  | 261/186/105/129/73     | 8.42            | 205.1214 | -4.4 |
| M7    |  | C <sub>13</sub> H <sub>16</sub> O <sub>2</sub> | 58753-01-8   | cyclohexanol-                 | 204.26    | 2.01                 | ± 0.23               | 18.161                            | 1.158       | 186/105/77  |                      |          | 17.167                                  | 276                  | 261/186/105/129/73     | 9.12            | 205.1216 | -3.4 |
| M8    |  | C <sub>13</sub> H <sub>16</sub> O <sub>2</sub> | 224426-74-8  | cyclohexanol-                 | 204.26    | 1.96                 | ± 0.23               | 18.226                            | 1.162       | 186/105/77  |                      |          | 17.353                                  | 276                  | 261/186/105/129/73     | 7.59            | 205.1215 | -3.9 |
| M9    |  | C <sub>13</sub> H <sub>16</sub> O <sub>2</sub> | 947-19-3     | cyclohexanol-                 | 204.26    | 2.18                 | ± 0.28               | 18.397                            | 1.173       | 186/105/77  |                      |          | not derivatized due to steric hydratace |                      | 10.3                   | 205.1213        | -4.9     |      |

\*log K<sub>ow</sub> values obtained from Sci Finder (ACD lab software)  
Unknown unsaturation position for M1 and M2

**Figure 1. Approach to characterization of main rainbow trout liver slice metabolites of cyclic phenones.**

## I. Metabolite Preparation: DPK, CPK and CBP Stds in EtOH

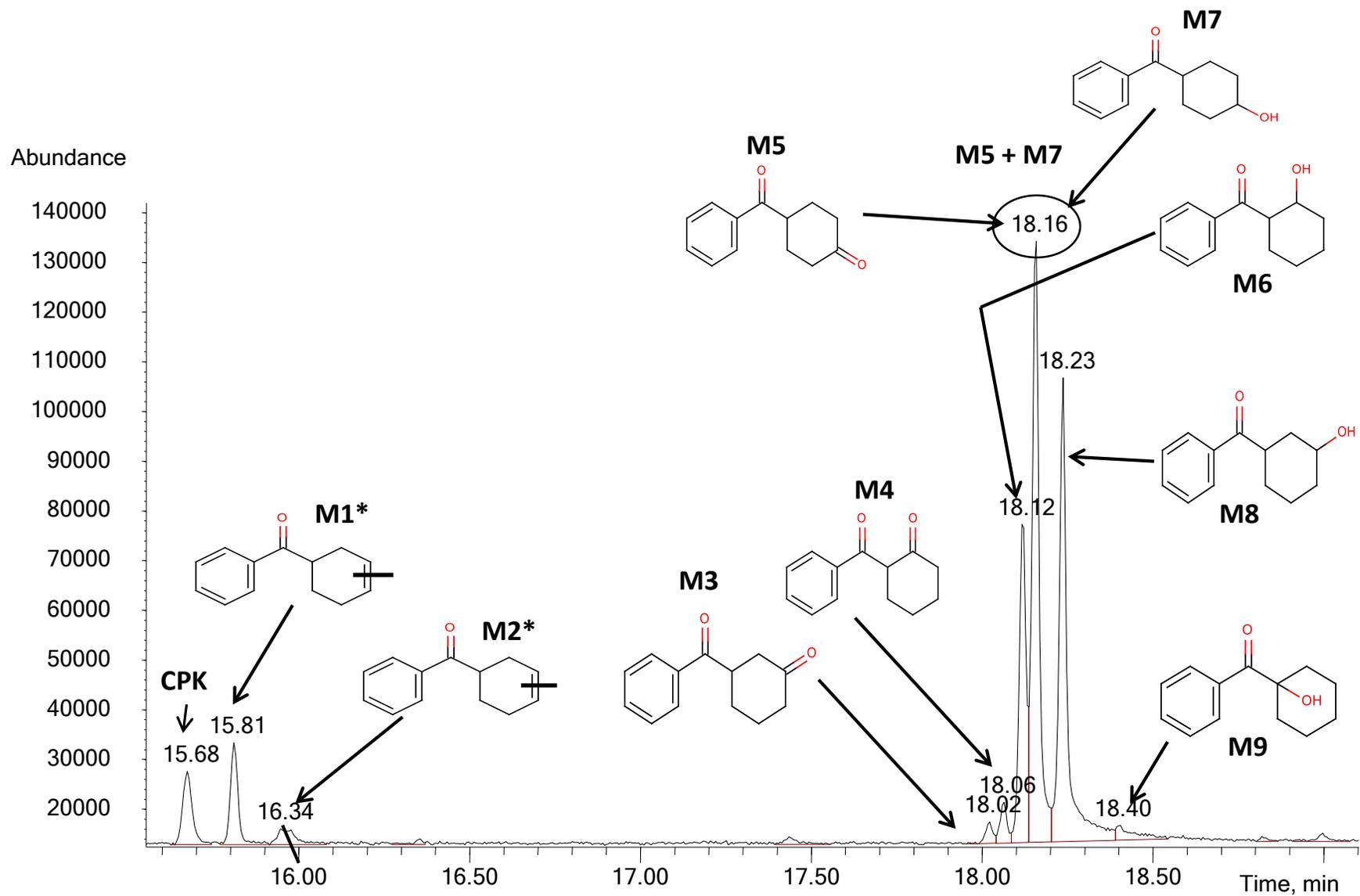
## II. Metabolite Characterization Studies



\*Identified with Stds by both GC- and LC-MS; no derivatization

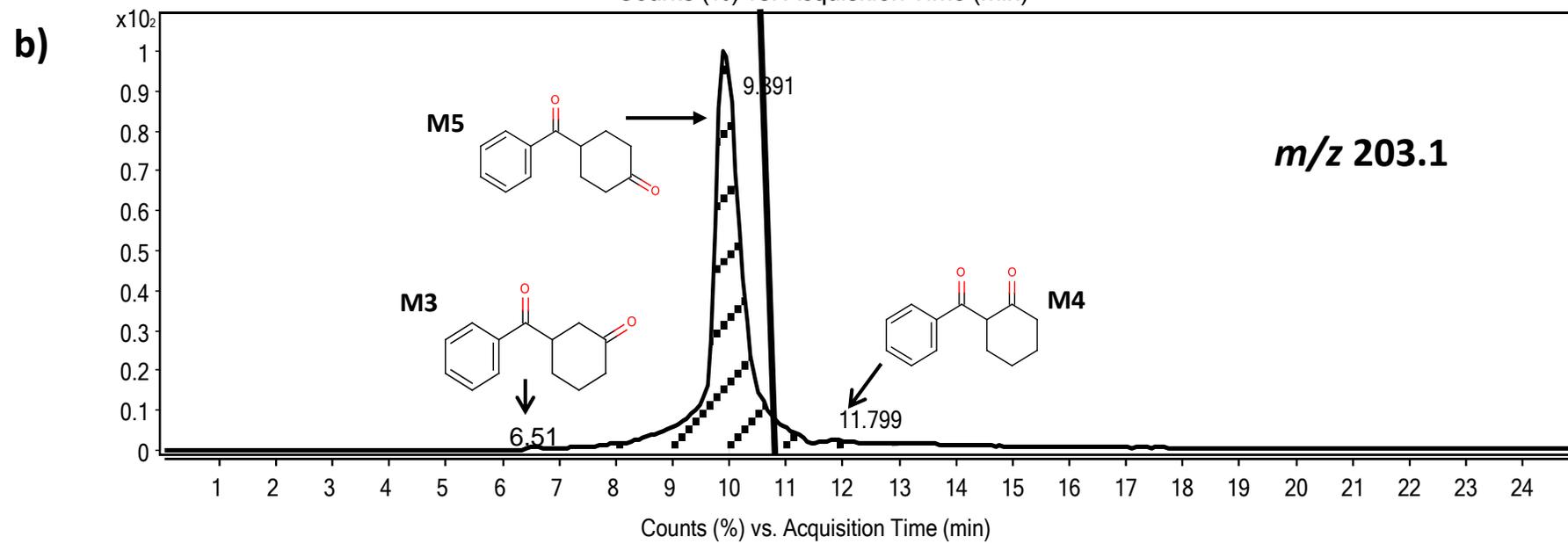
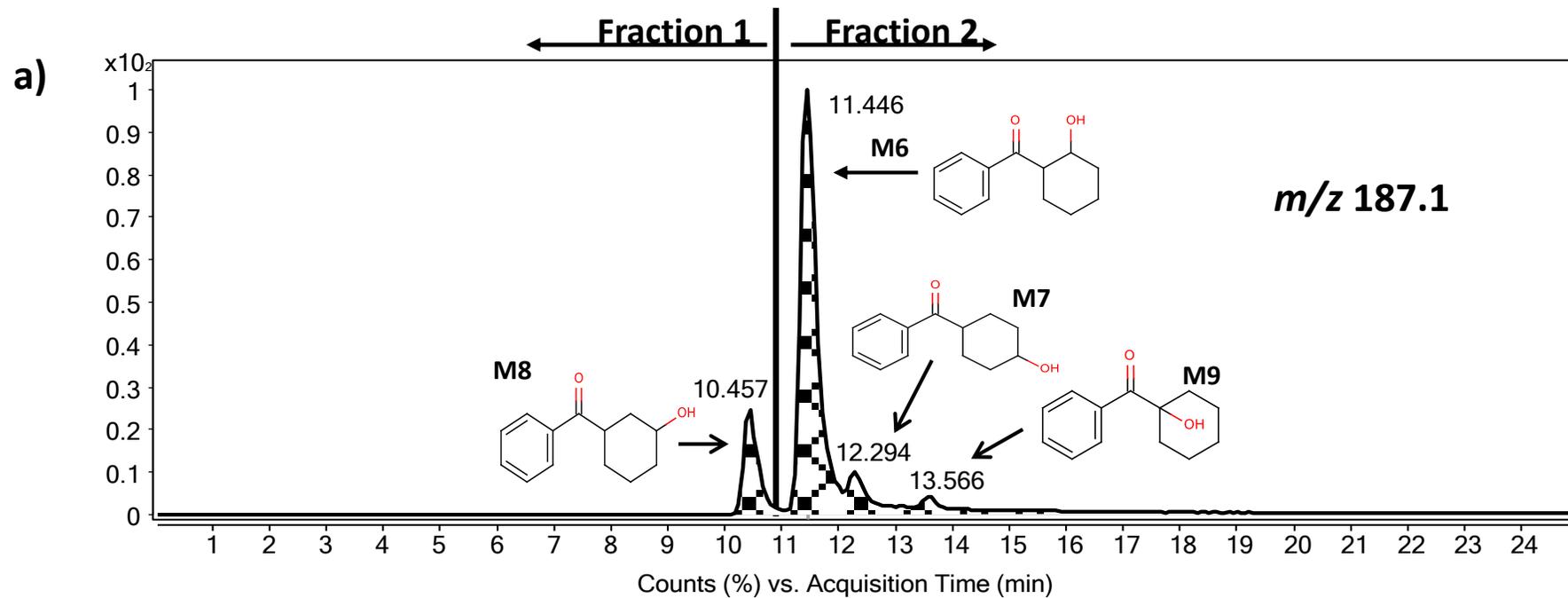
‡Identified by GC-MS data only; no derivatization

**Figure 2. GC-EI-MS total ion chromatogram (TIC) of CPK and main metabolites, and their proposed structures.**

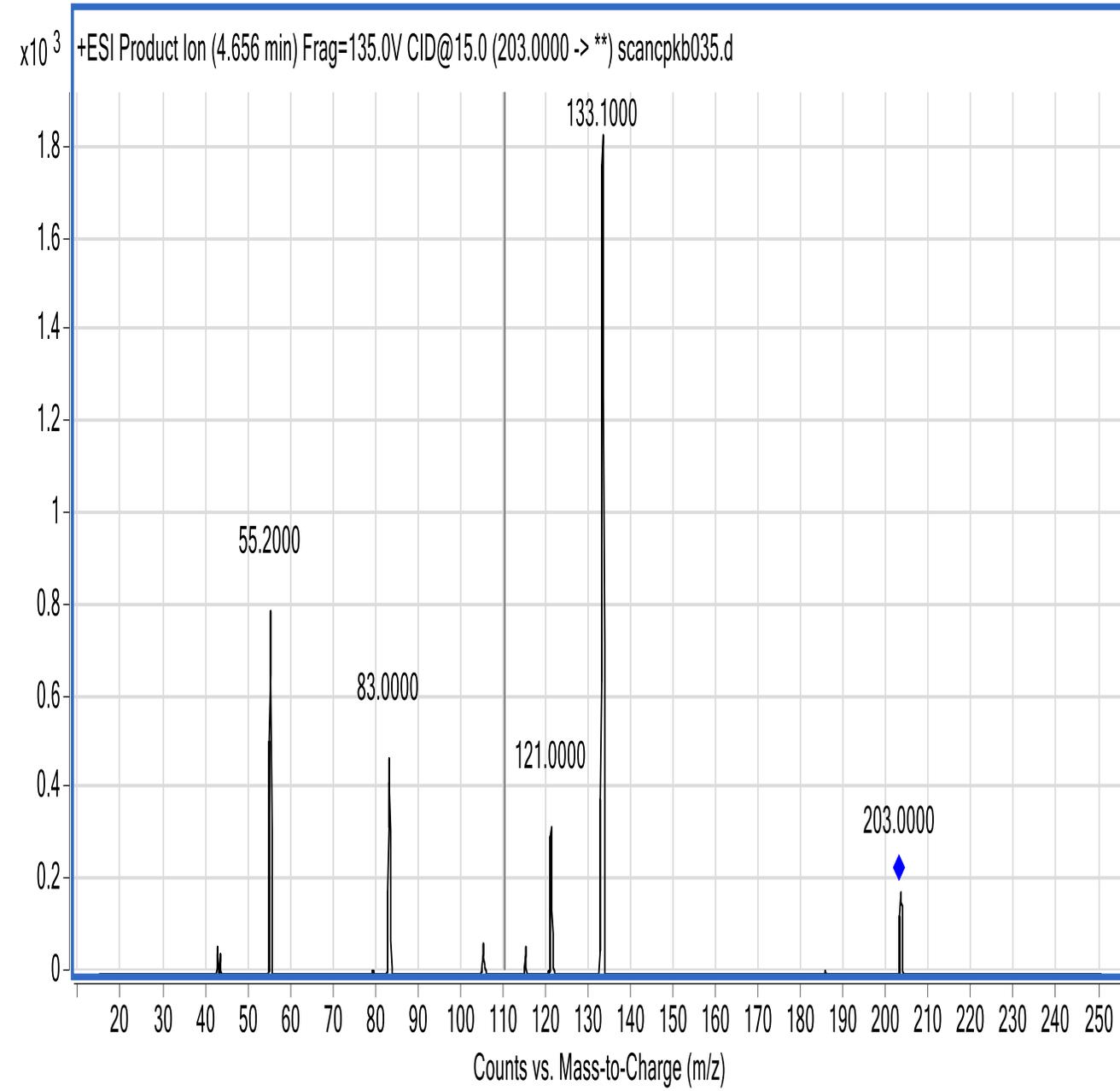
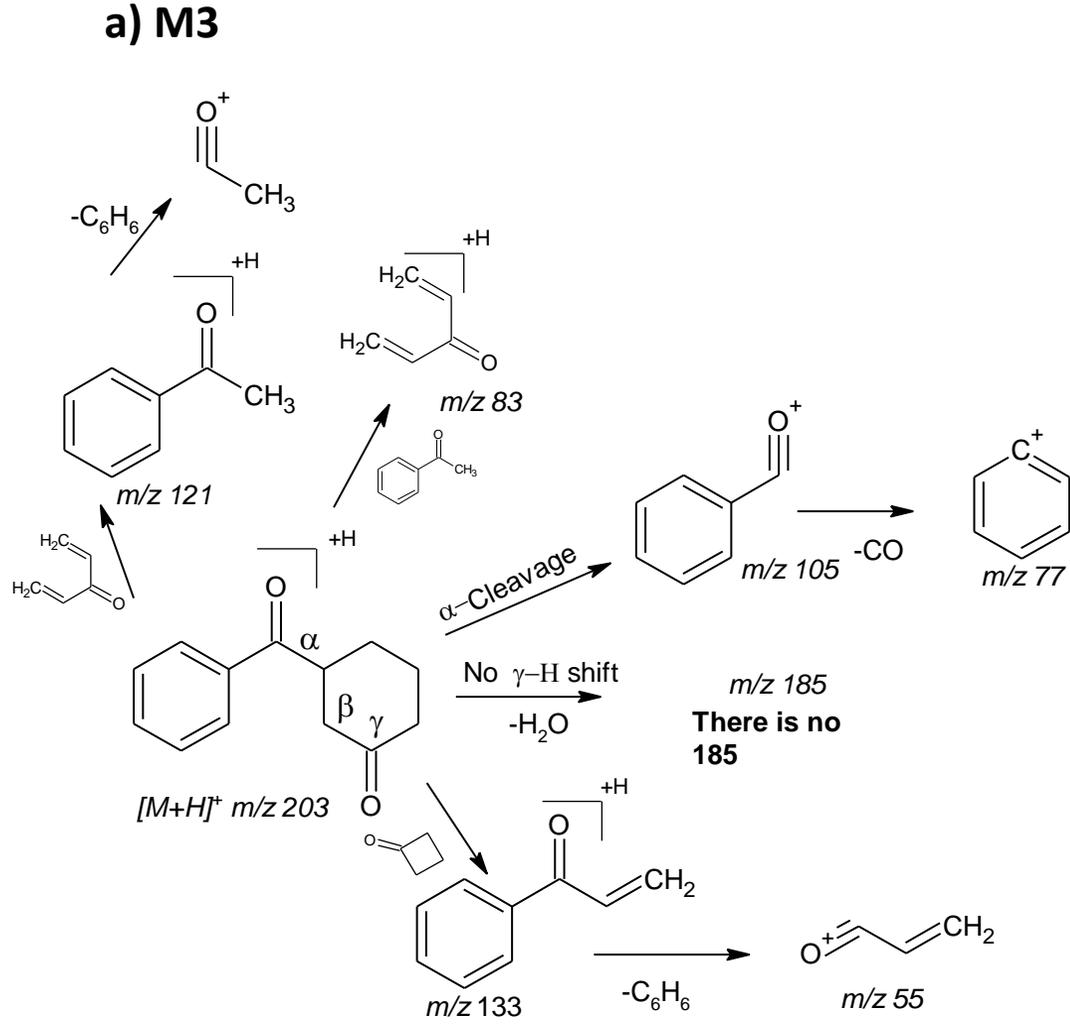


\* Unknown unsaturation position

**Figure 3. LC-TOF-MS extracted ion chromatograms (EIC) of CPK metabolites: a) EIC of  $m/z$  187.11 (M6-M9); b) EIC of  $m/z$  203.11 (M3-M5)**

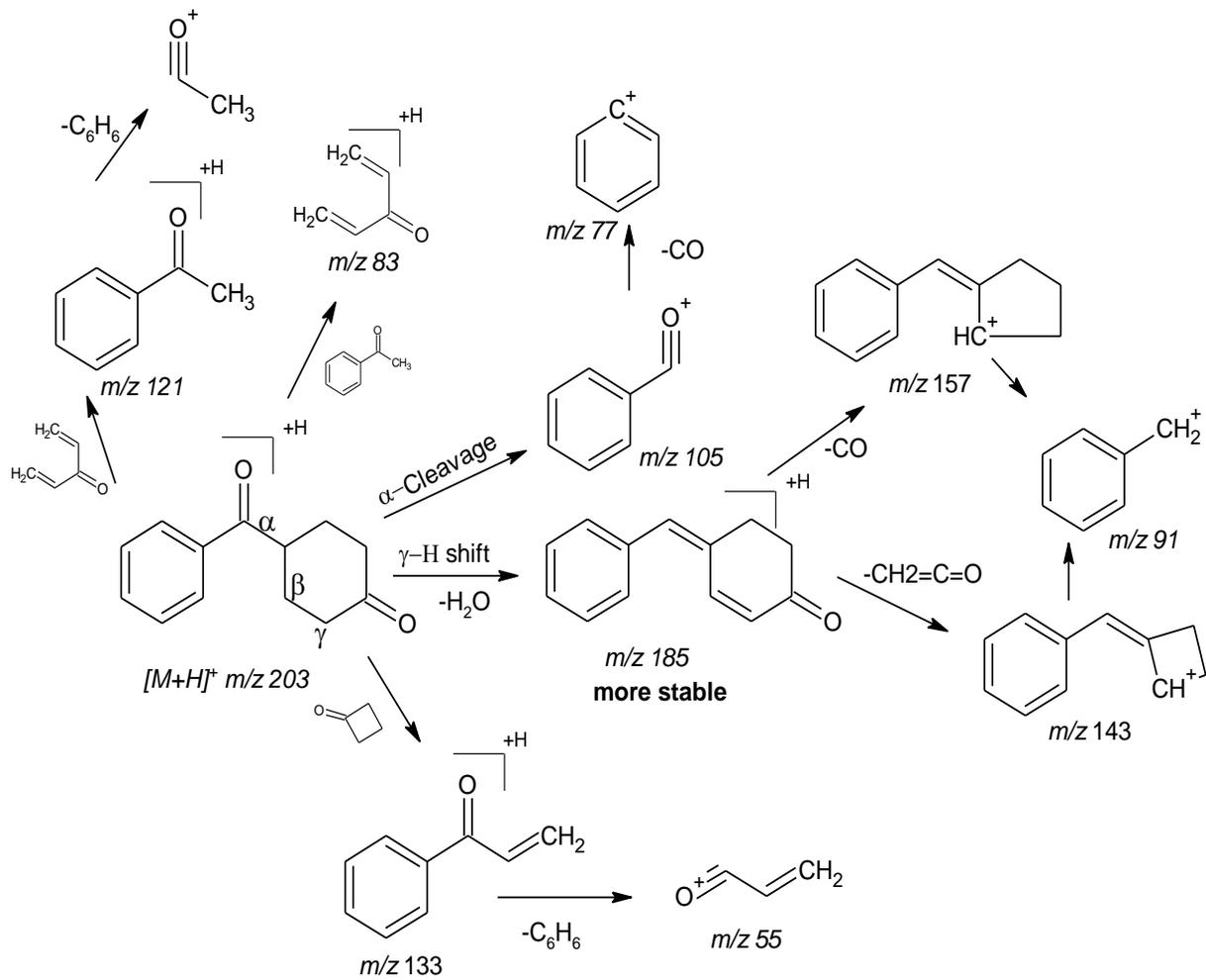


**Figure 4. LC-MS/MS product ion mass spectra of [M+H<sup>+</sup>] of *m/z* 203 and proposed fragmentation pathway for a) M3, b) M4 and c) M5 metabolites.**

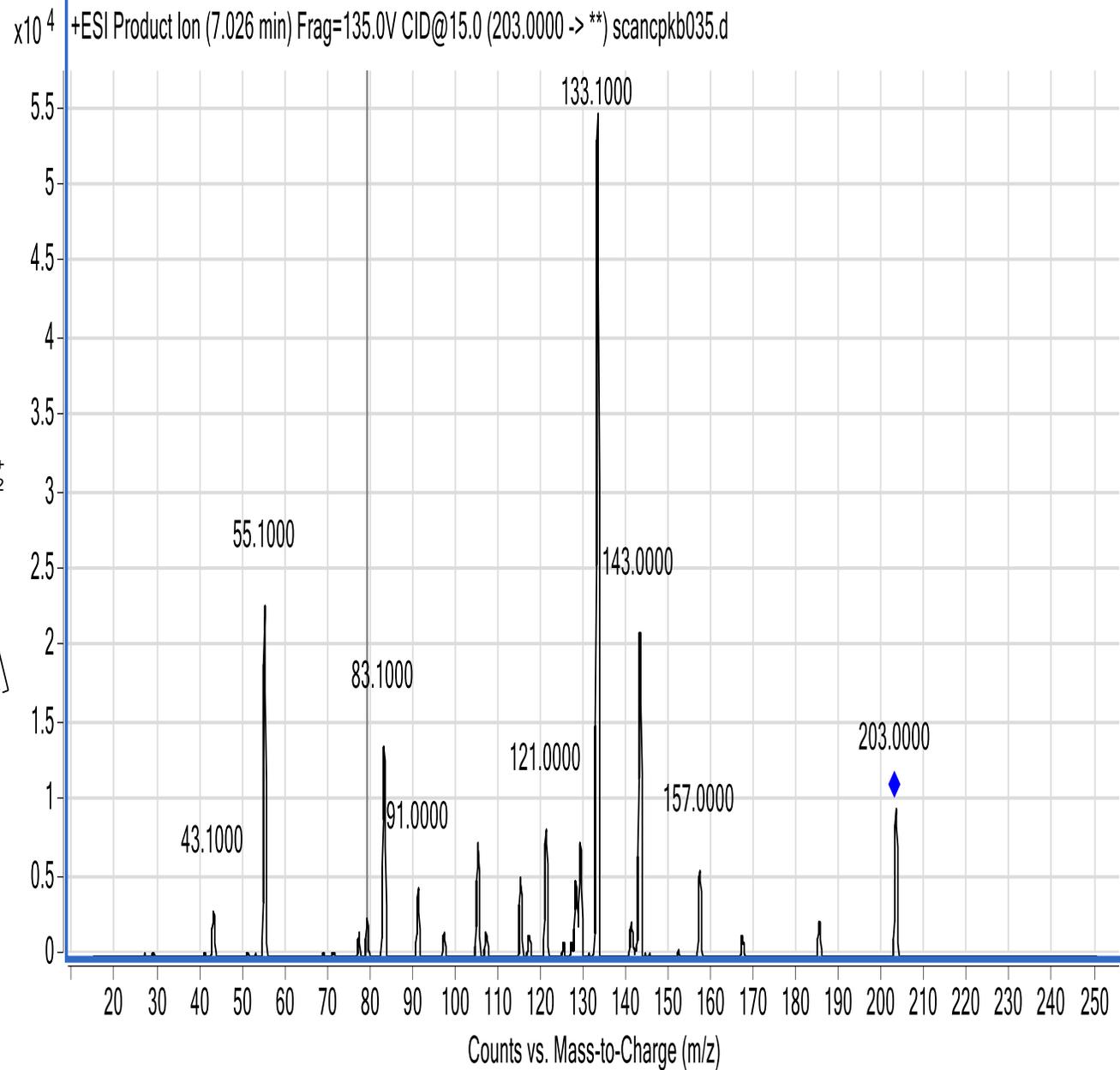




### c) M5

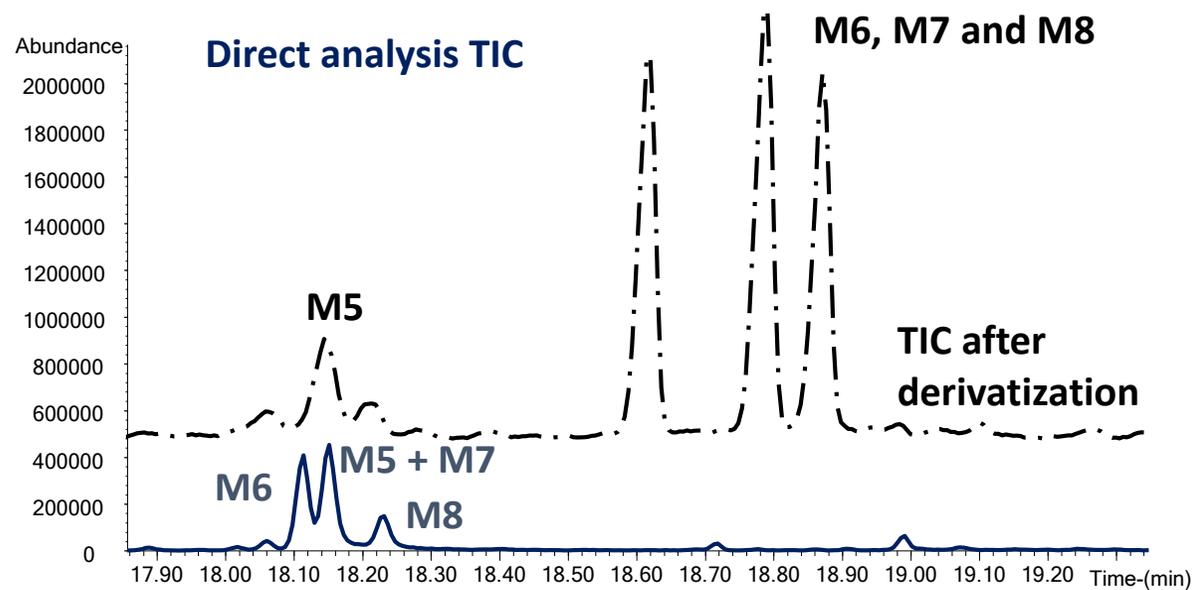


+ESI Product Ion (7.026 min) Frag=135.0V CID@15.0 (203.0000 -> \*\*) scancpkb035.d

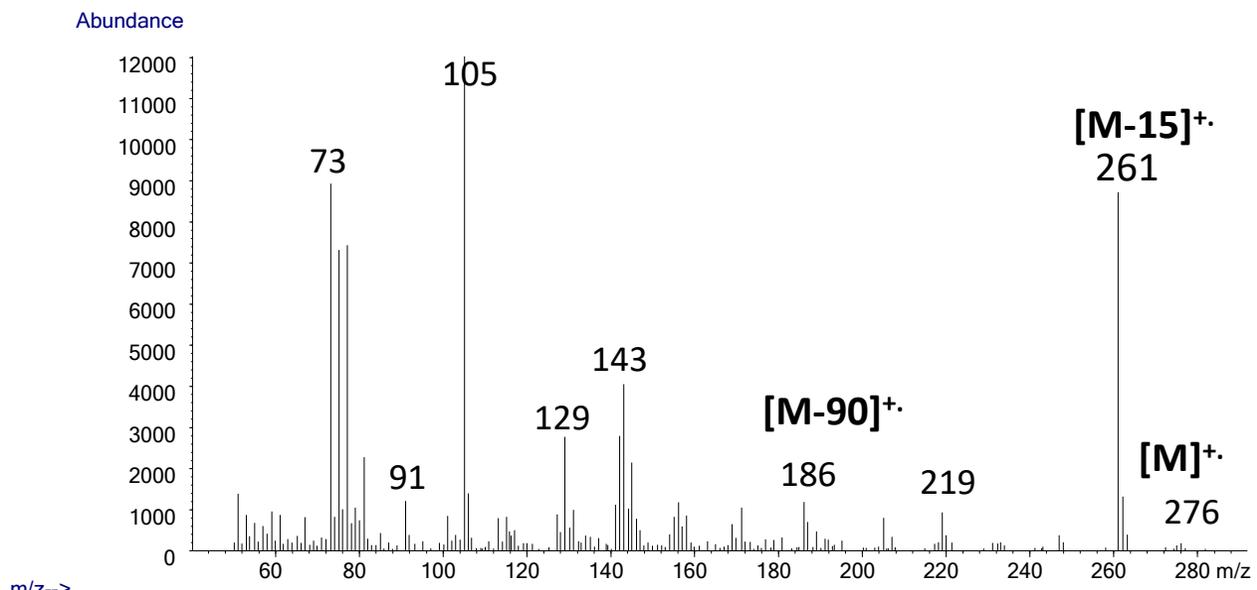


**Figure 5. Direct GC-MS analysis of CPK metabolites with and without BSTFA derivatization a) TIC chromatograms; b) representative mass spectrum of derivatized metabolites (M6-M8)**

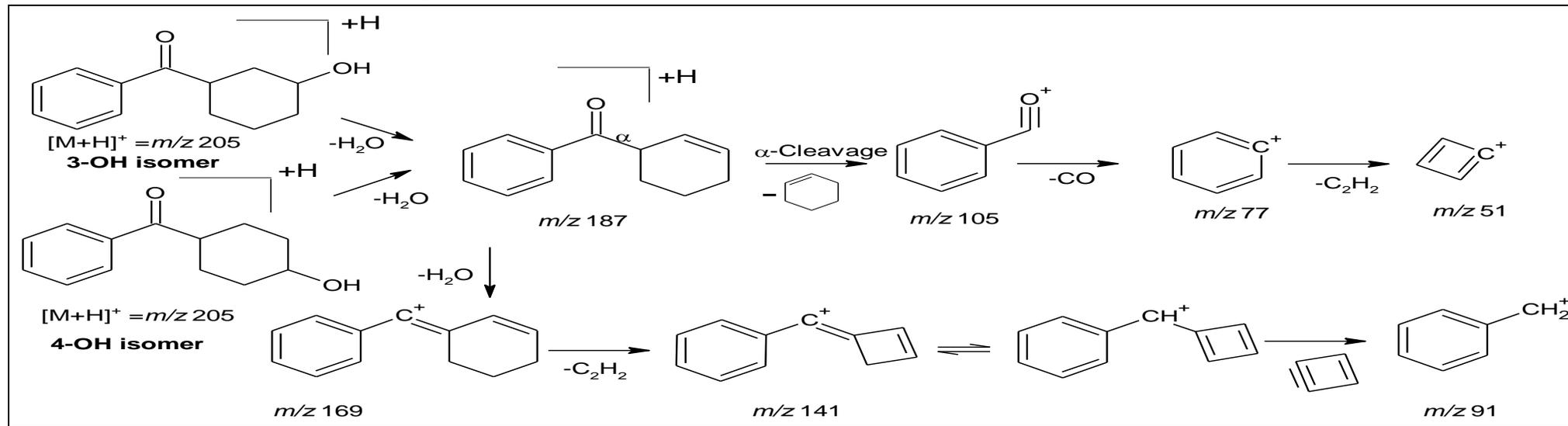
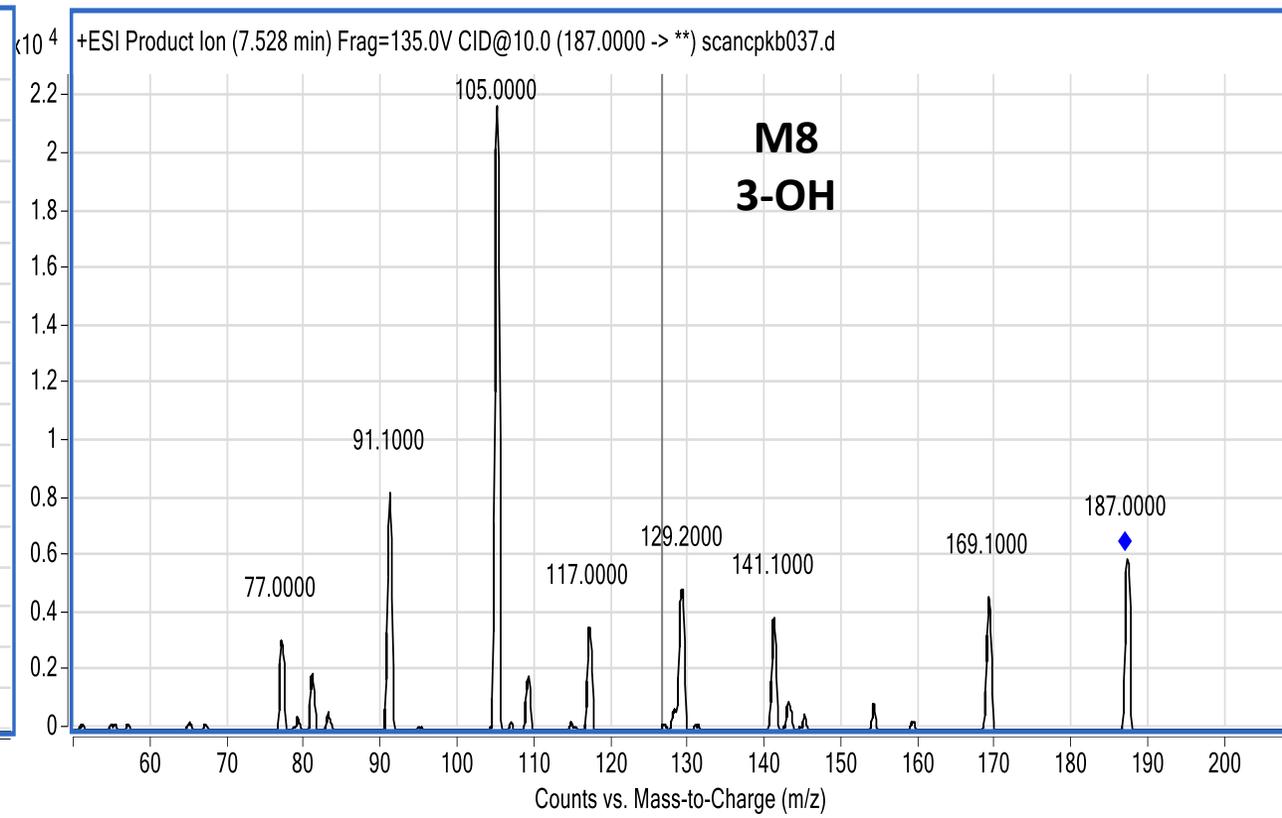
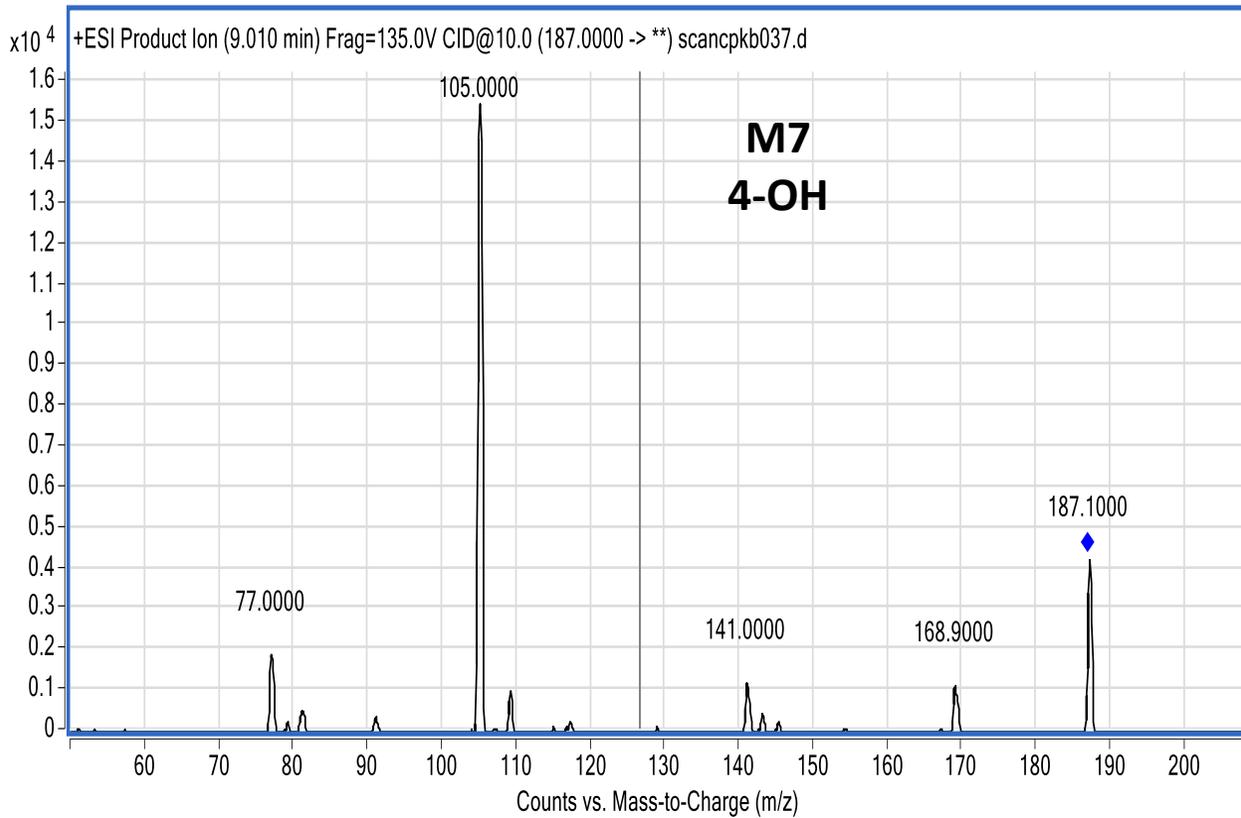
a)



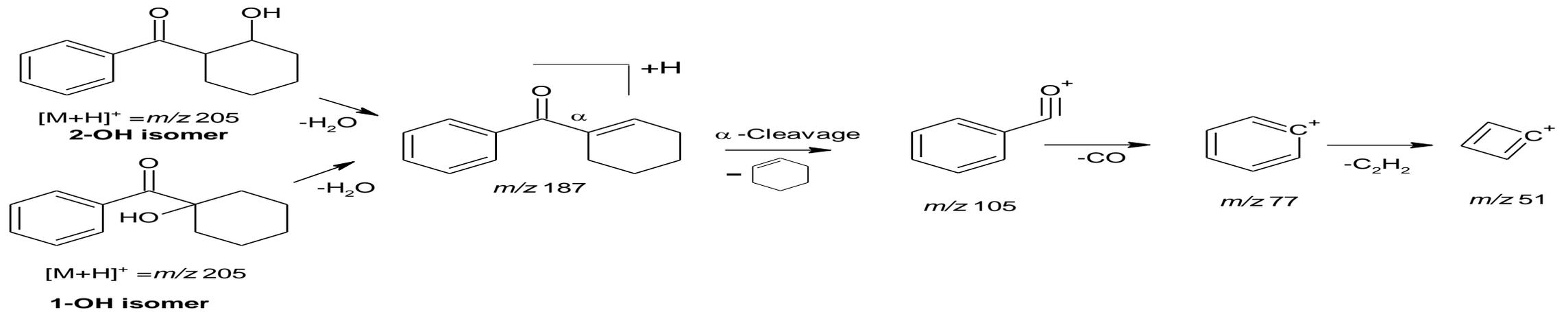
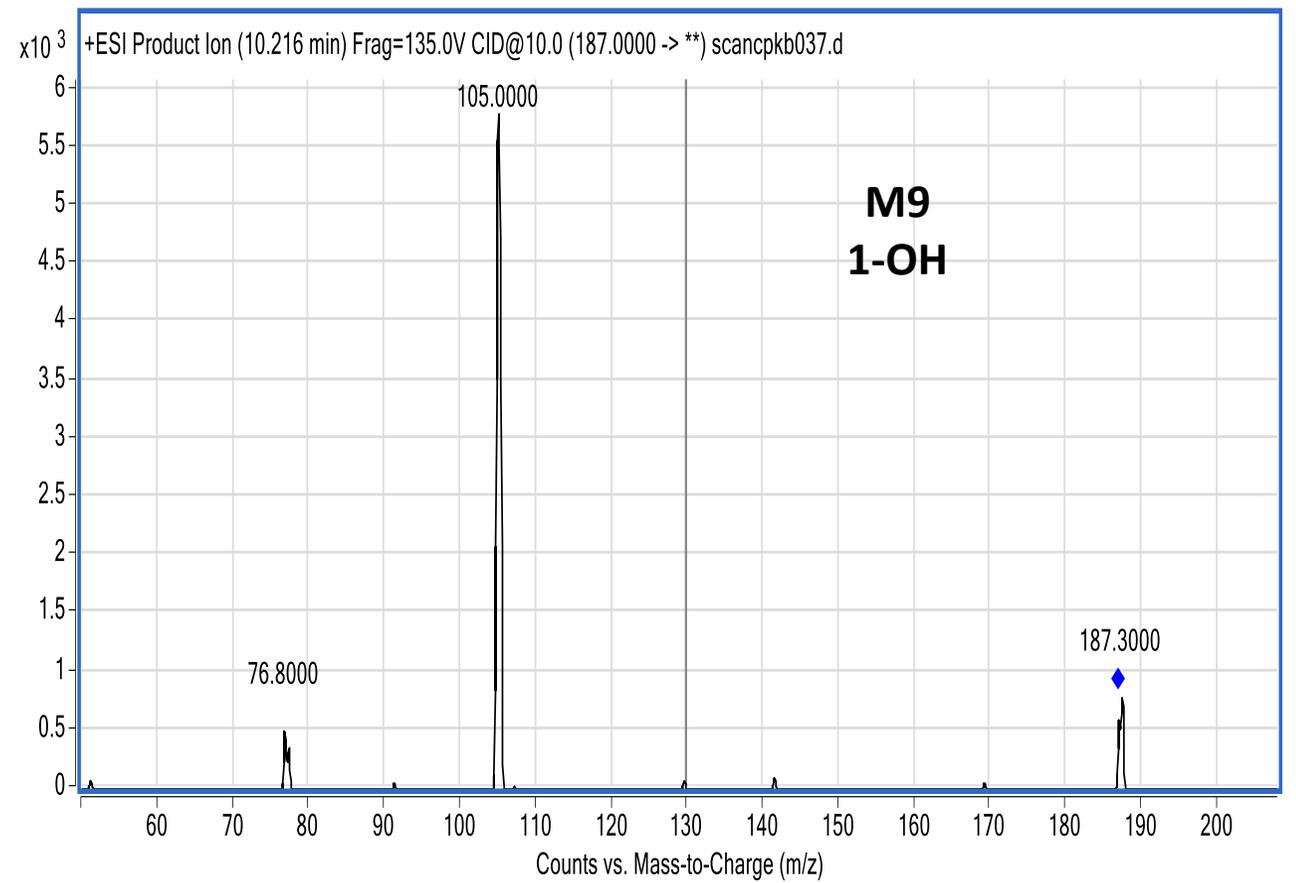
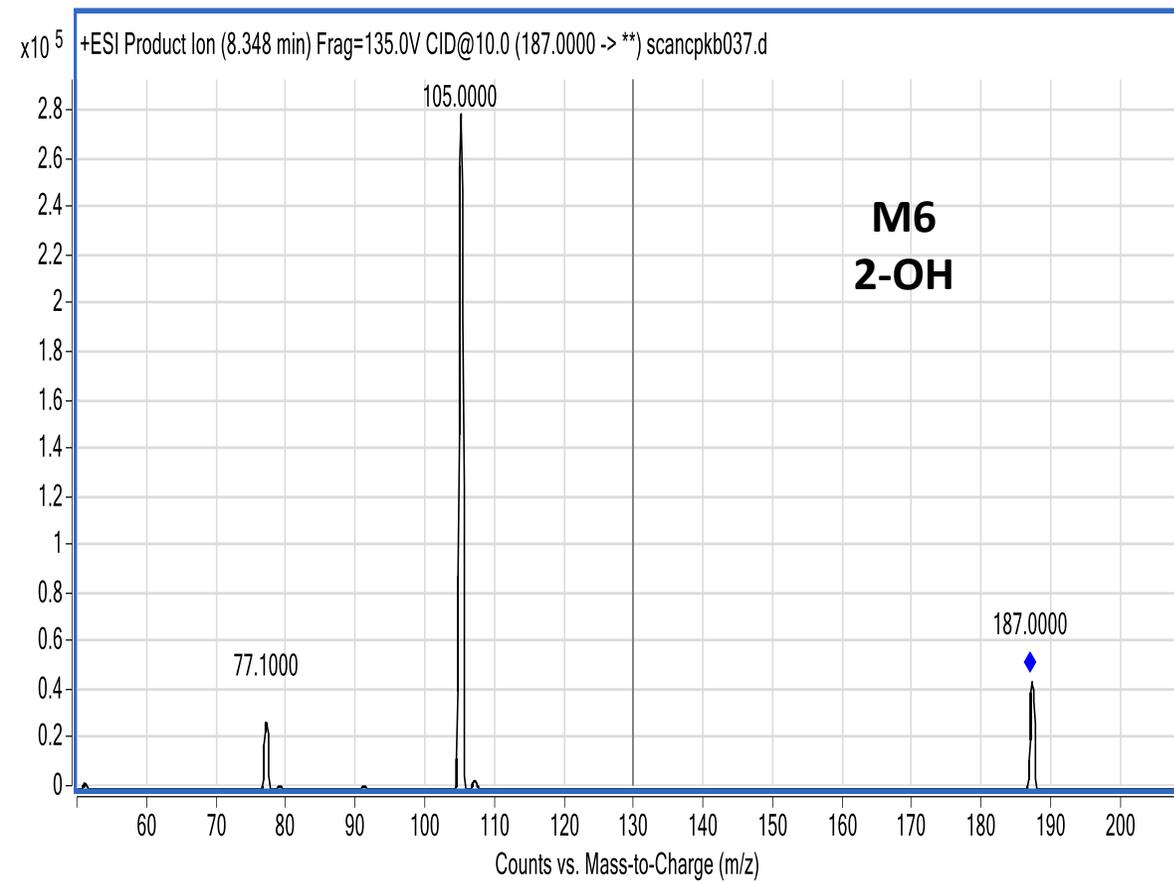
b)



**Figure 6. LC-MS/MS data and proposed fragmentation pattern for CPK Metabolites M7 and M8**

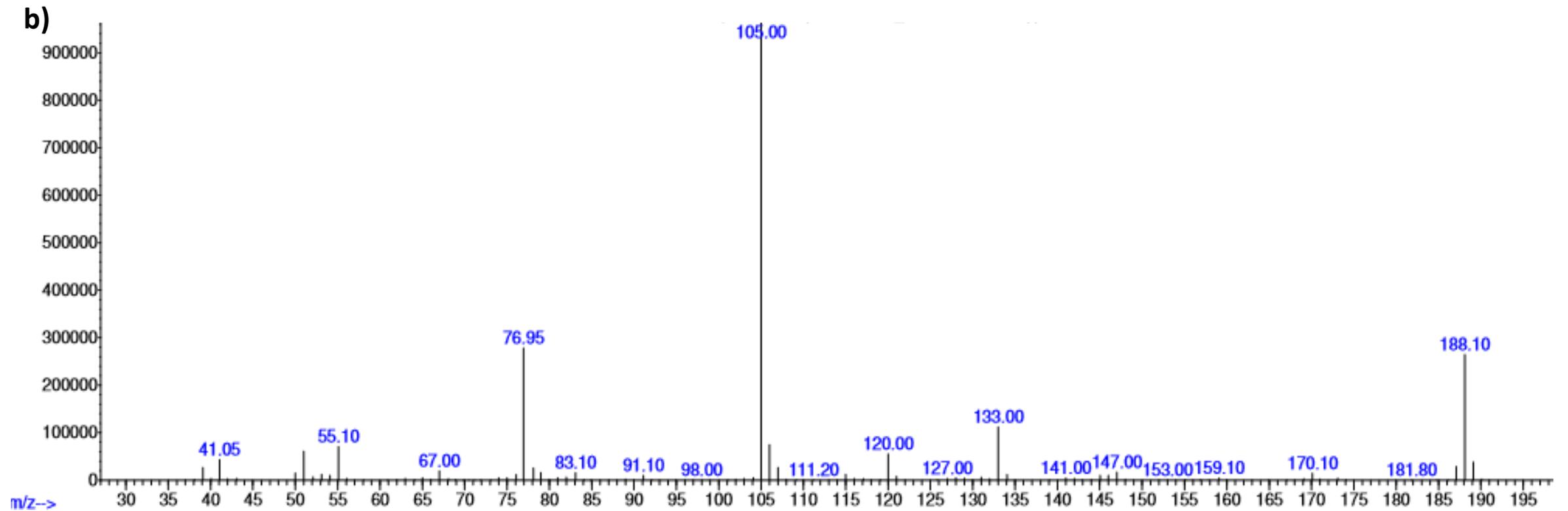
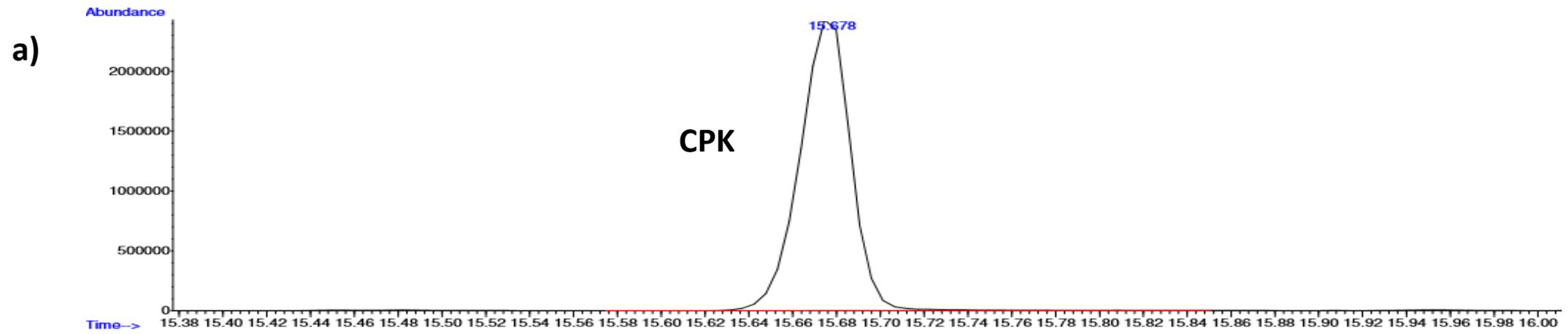


**Figure 7. LC-MS/MS data and proposed fragmentation pattern for CPK Metabolites M6 and M9**



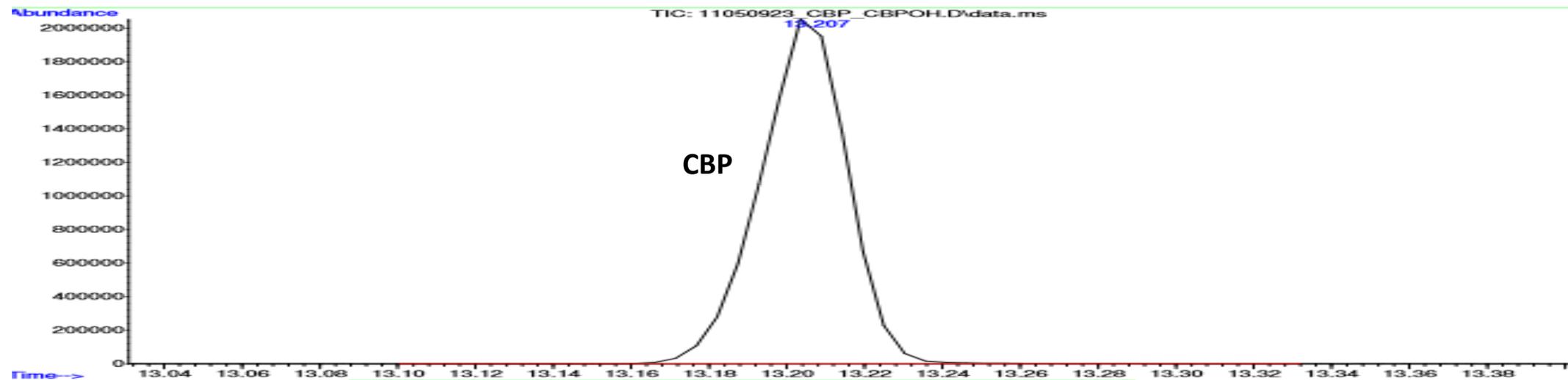
# **Supplemental Figures**

**Supplemental Figure 1. GC-EI-MS TIC (a) and representative mass spectrum (b) for CPK Standard ( $m/z=188$ )**

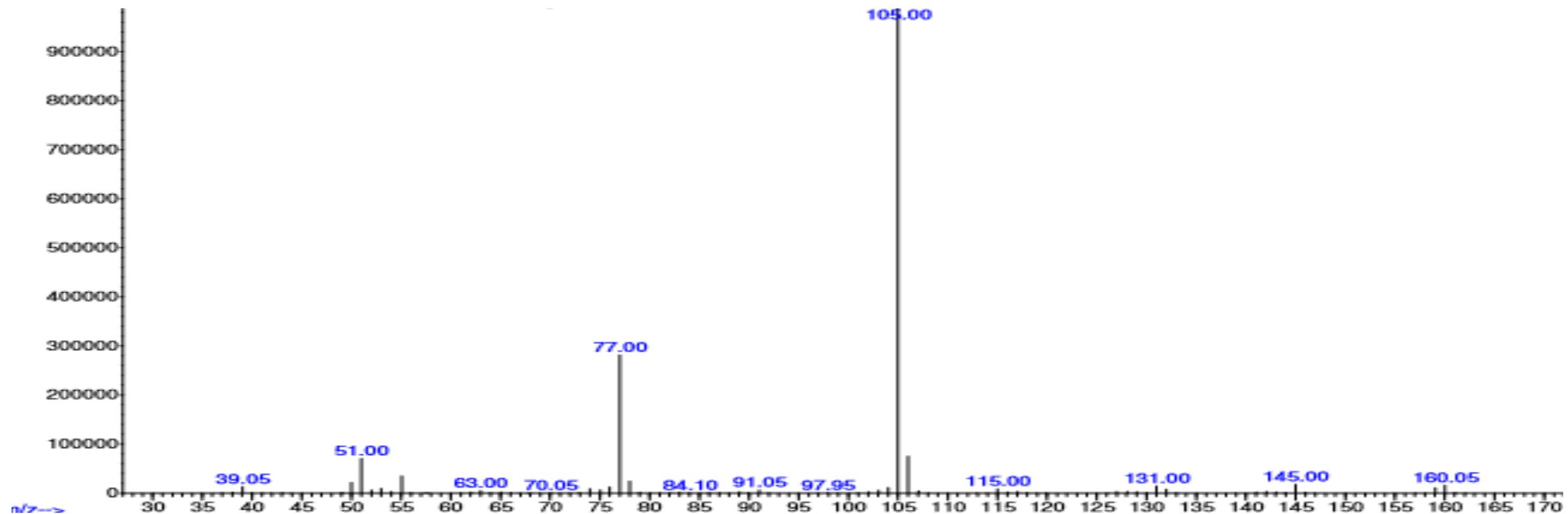


**Supplemental Figure 2. GC-EI-MS TIC (a) and representative mass spectrum (b) for CBP Standard ( $m/z=160$ )**

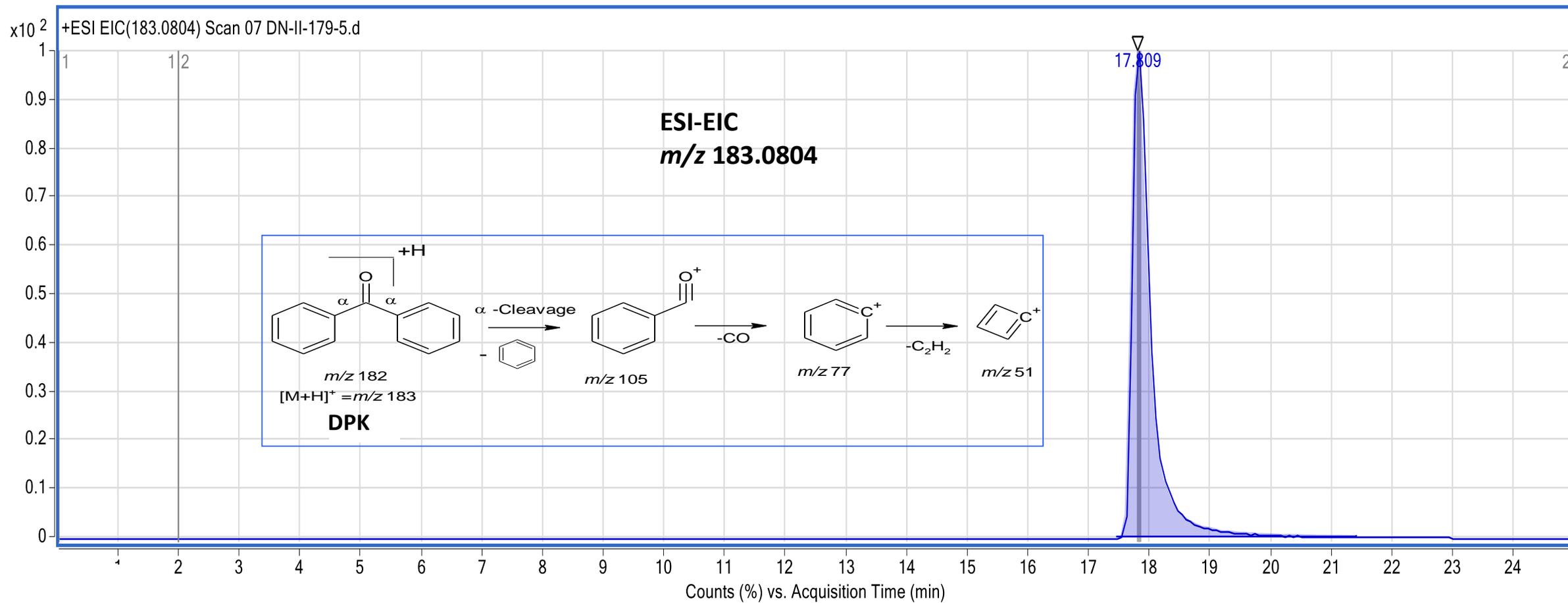
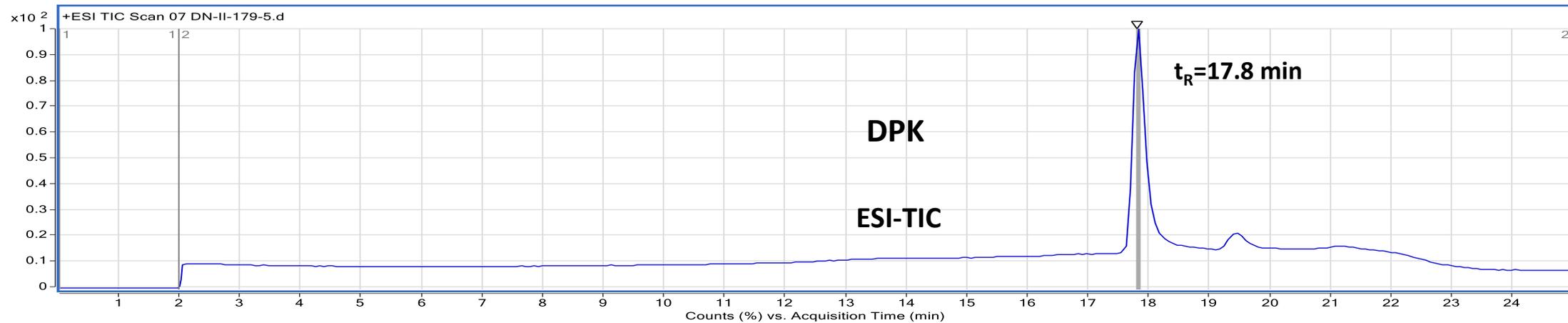
a)



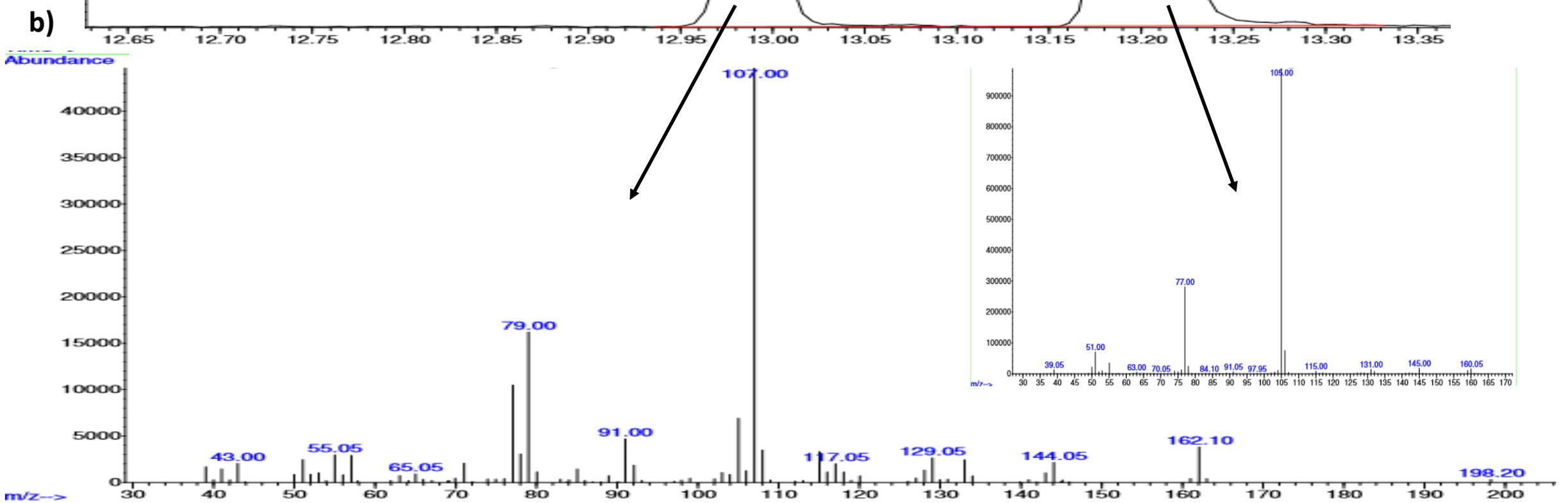
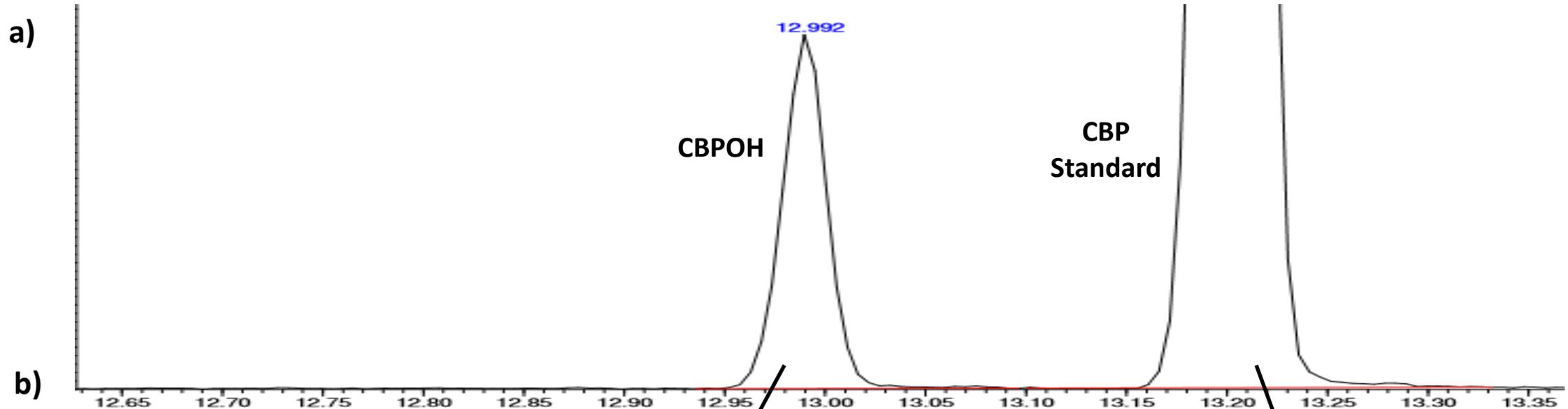
b)



**Supplemental Figure 3. LC-ToF-MS of DPK Standard (extracted  $m/z$  183.0804)**

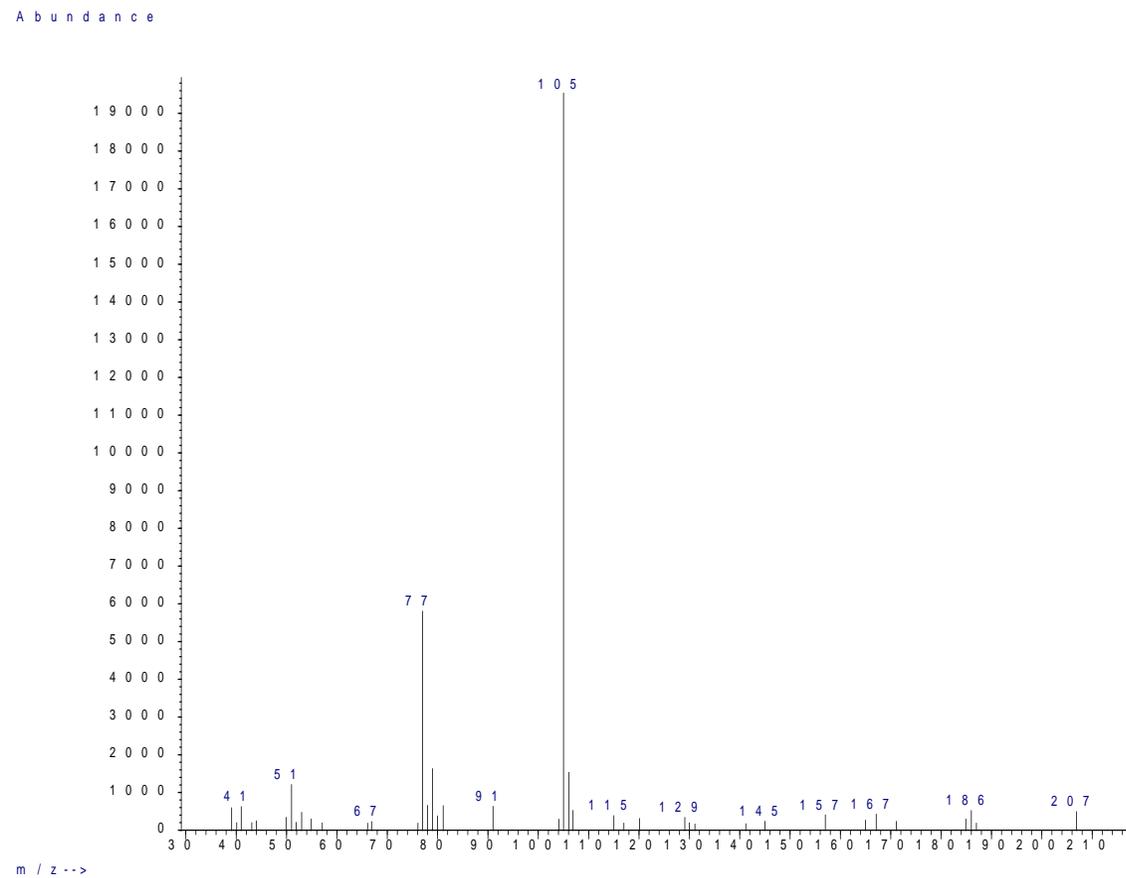


**Supplemental Figure 4. a) GC-EI-MS TIC and mass spectra (b) for CBP Standard and CBPOH metabolite prepared by CBP incubation with rt slices**

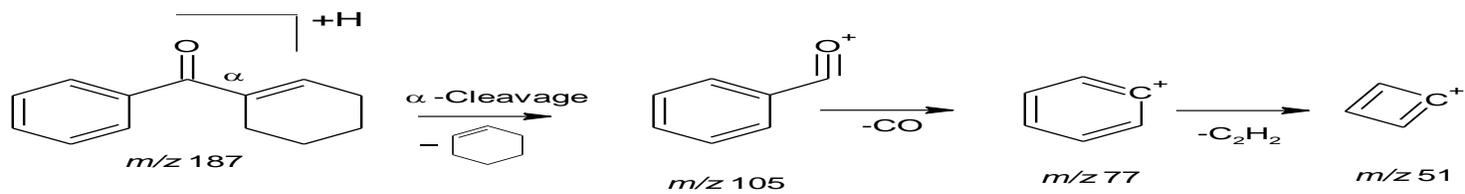
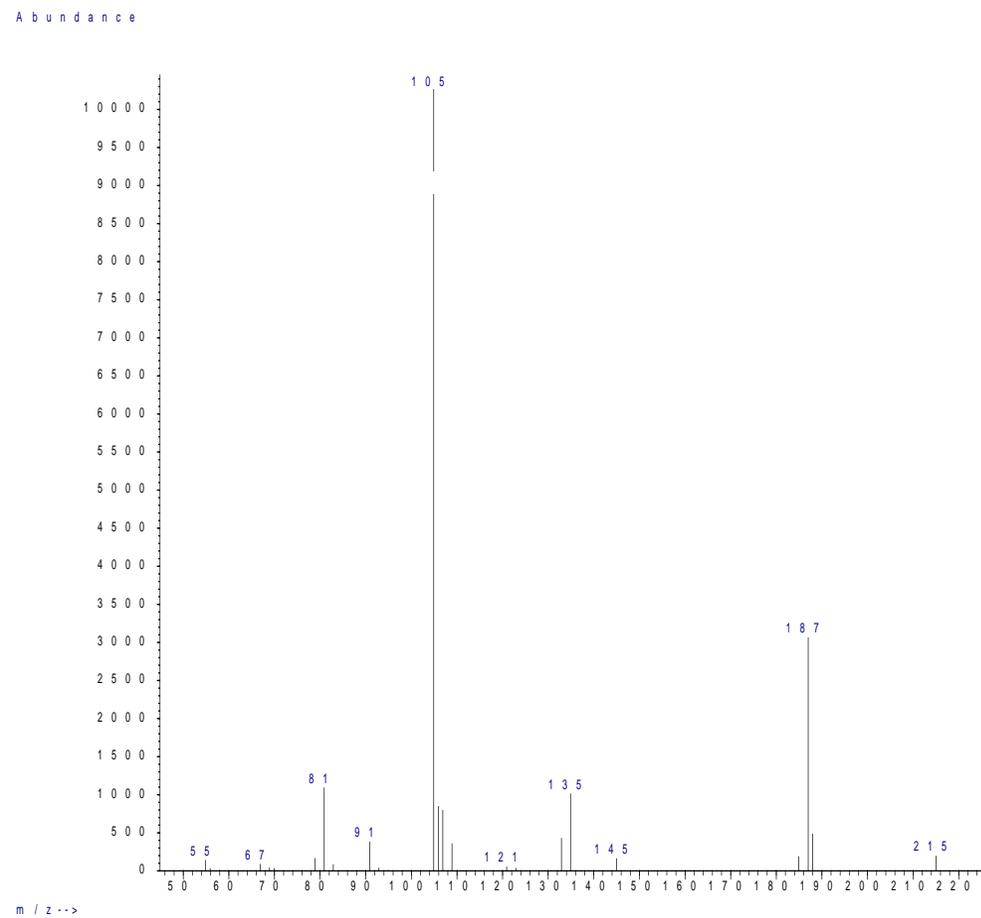


**Supplemental Figure 5a. a) GC-EI- MS and b) GC-CI-MS mass spectra of CPK Metabolite M1**

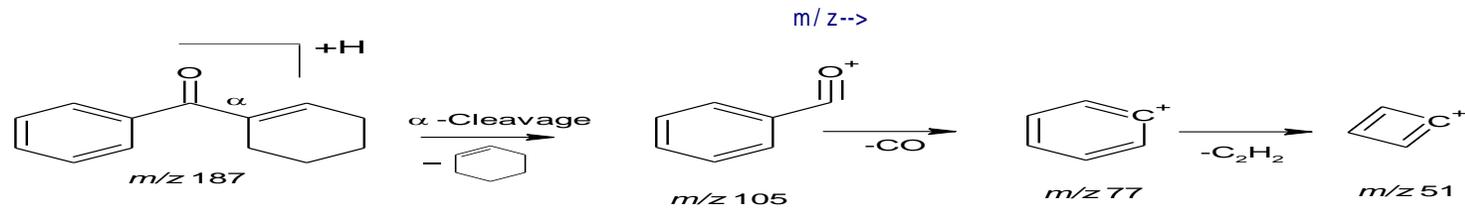
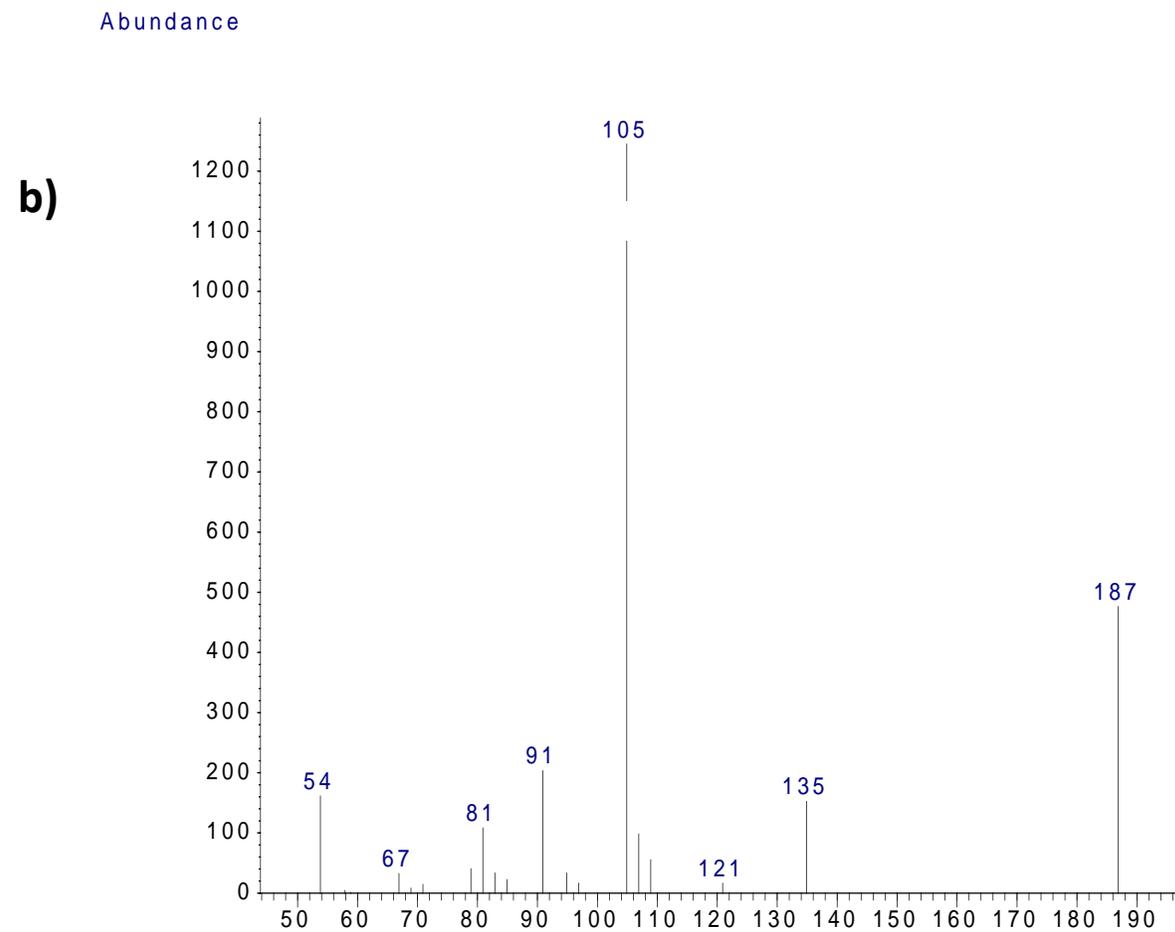
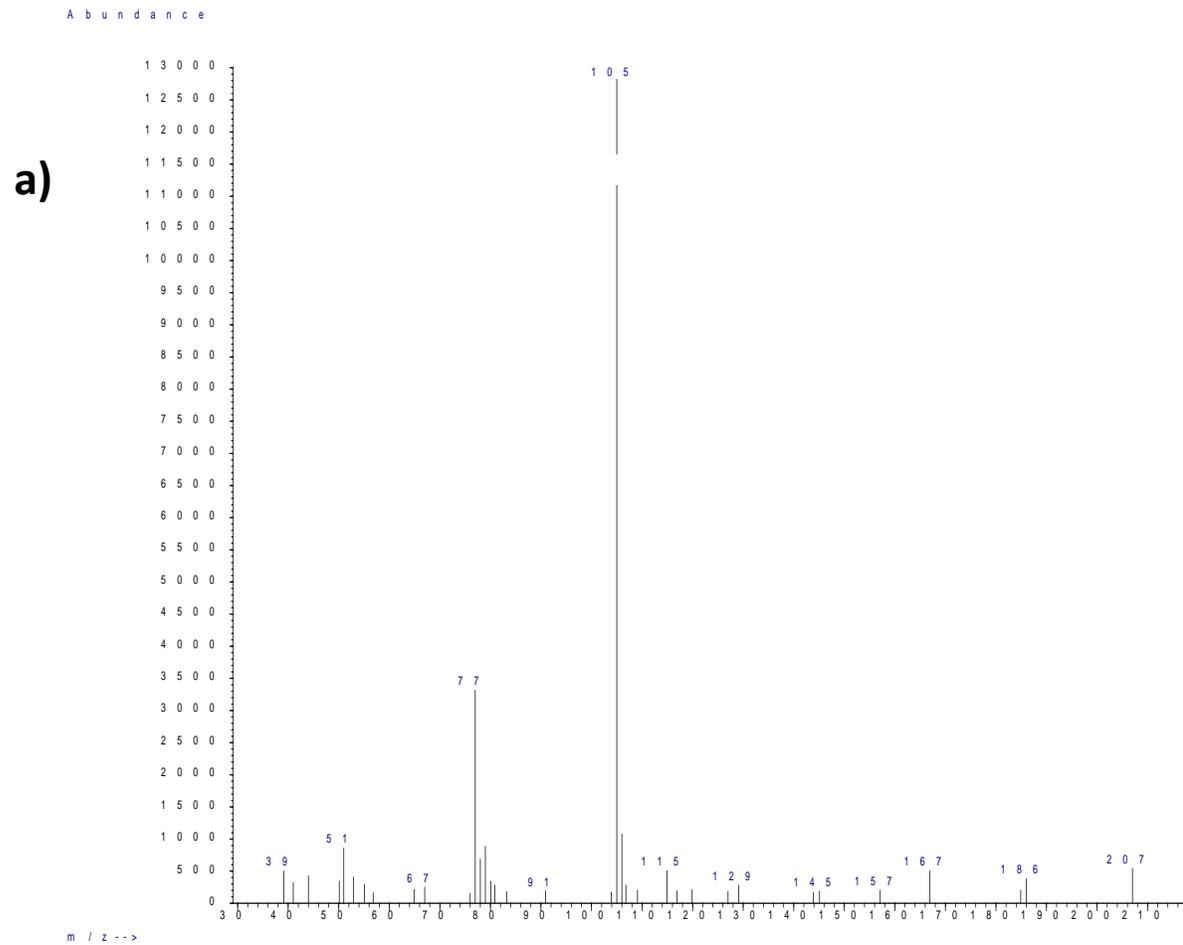
a)



b)



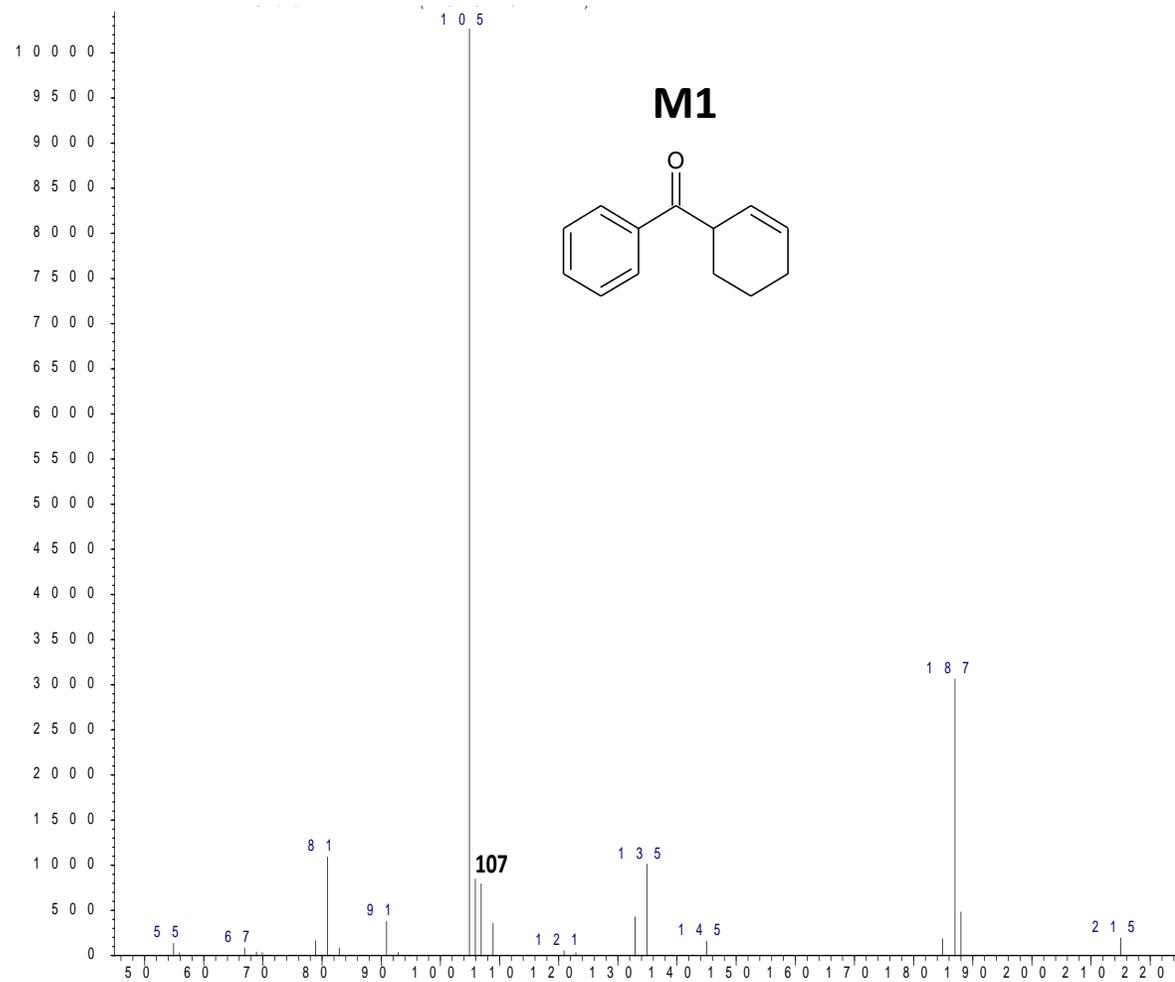
**Supplemental Figure 5b. a) GC-EI- and b) GC-Cl-MS mass spectra of CPK Metabolite M2**



**Supplemental Figure 6. Characteristic GC-Cl-MS fragmentation for CPK phenyl ketone metabolites M1 (a)  
and M3 (b)**

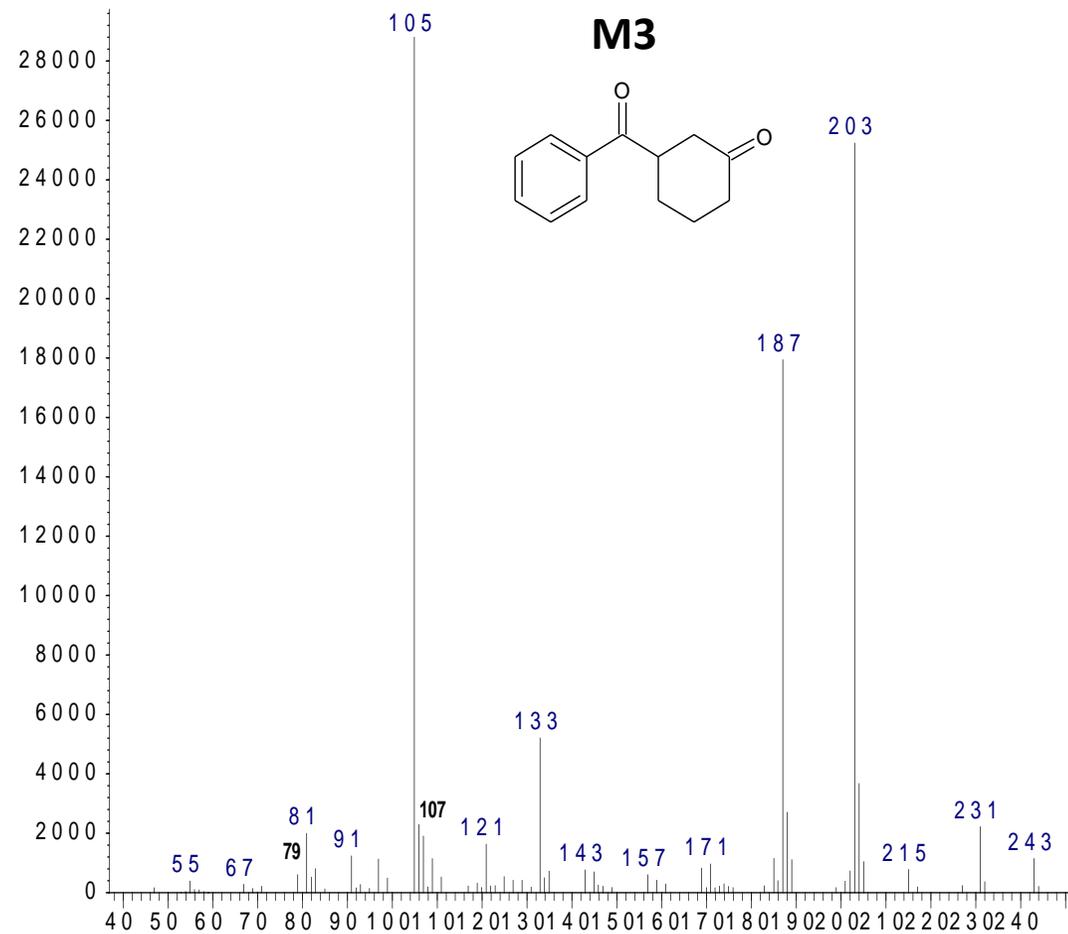
a)

Abundance



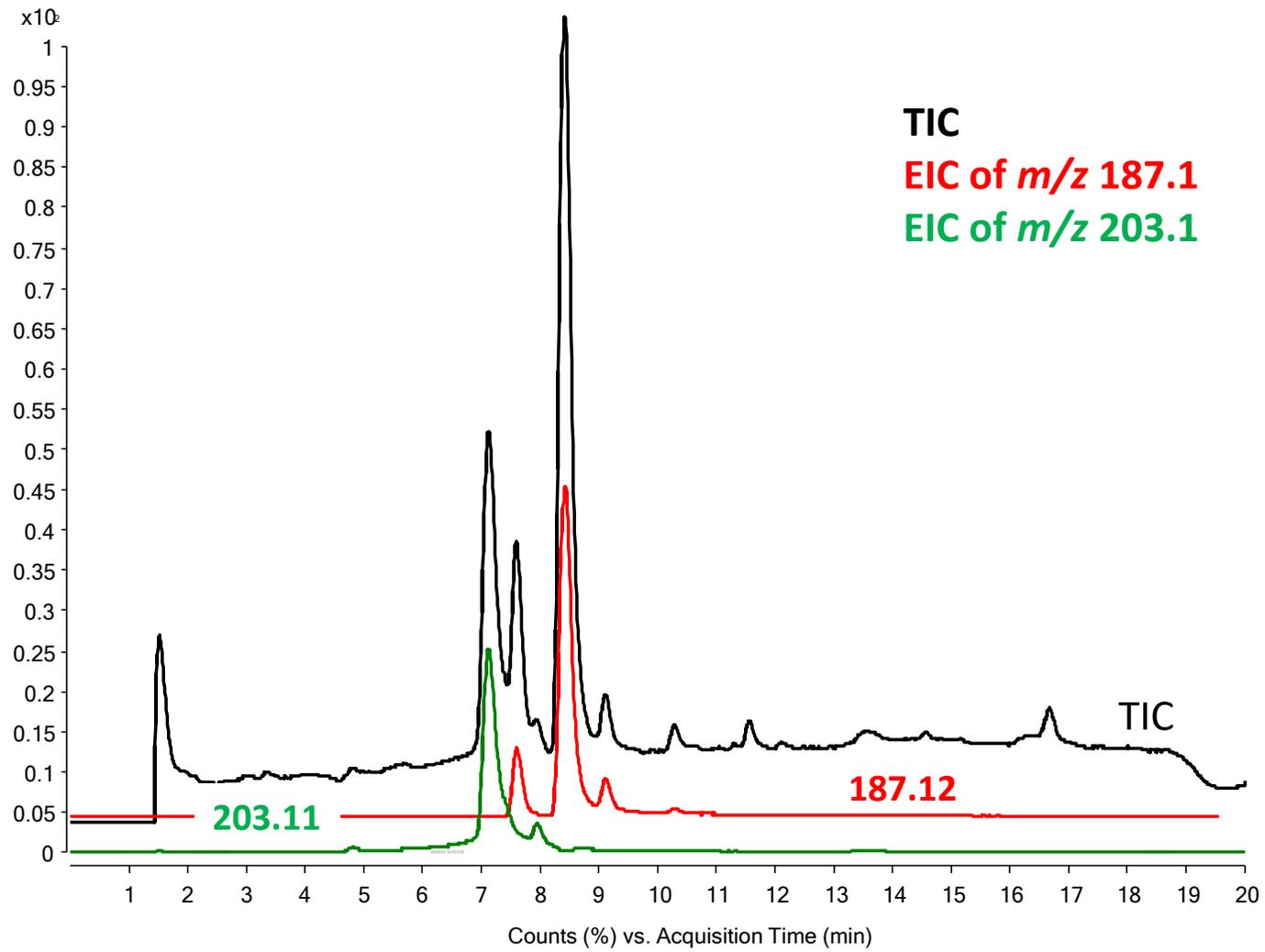
b)

Abundance



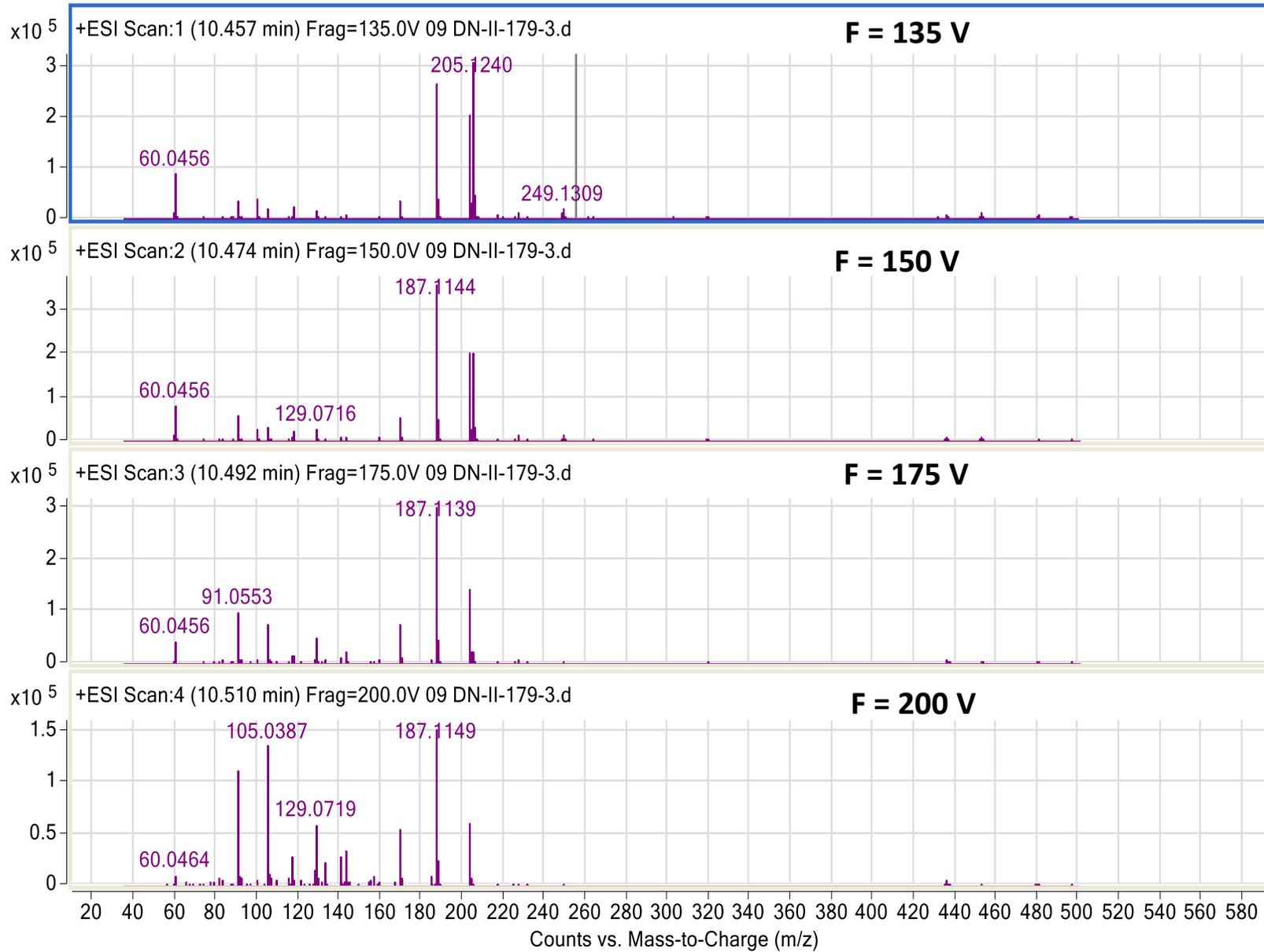
m/z-->

**Supplemental Figure 7. LC-MS total ion chromatogram (TIC) and extracted ion chromatograms (EIC) for CPK metabolites**



**Supplemental Figure 8a. LC-TOF-MS of CPK Metabolite M8 showing effect of fragmentation voltage variation on ion at  $m/z$  205 and stronger formation of ions at  $m/z$  169, 141 and 91.**

# M8

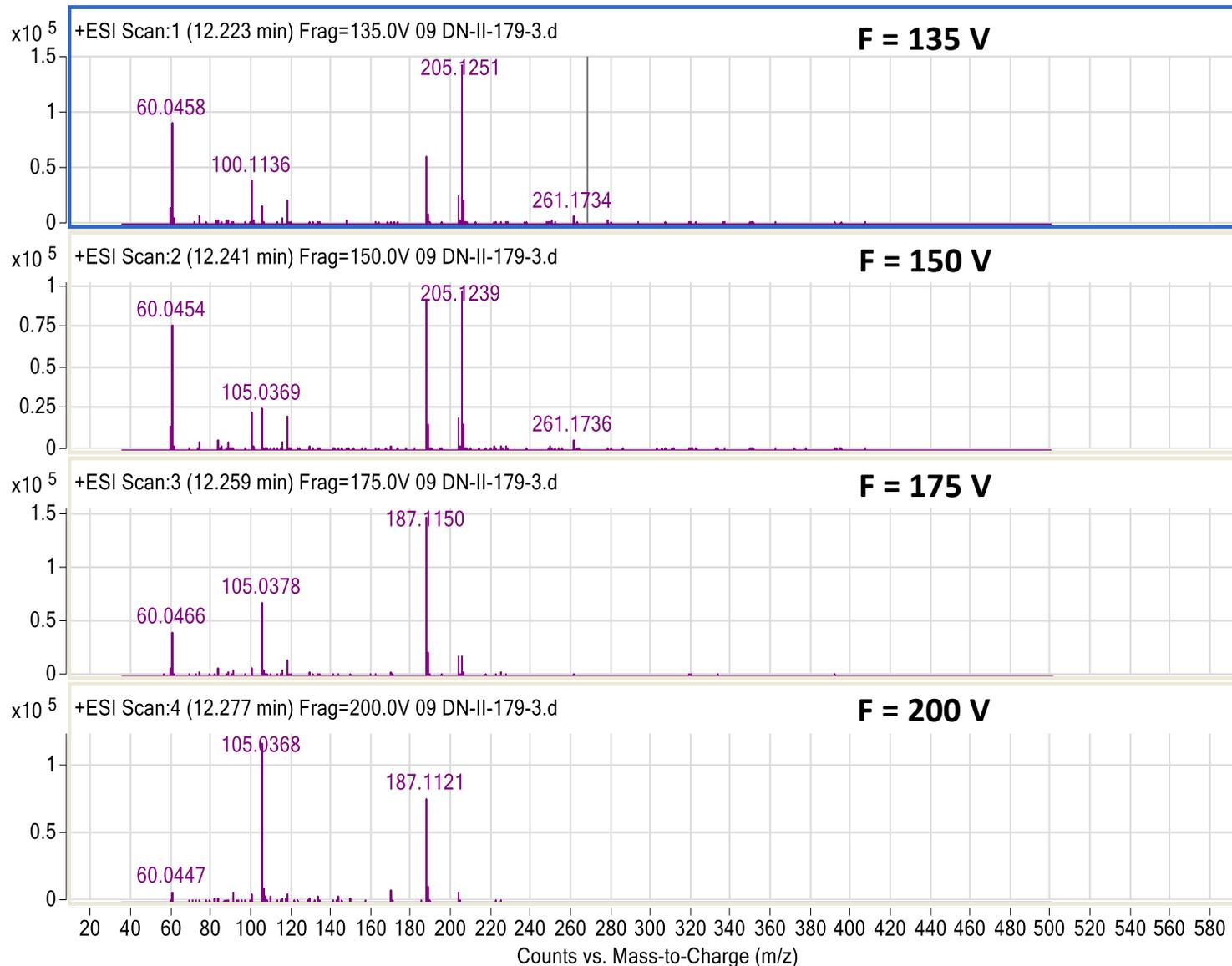


# M8

| Error (ppm) | Measured (m/z) | Calculated (m/z) |                | ESI/F (V) |
|-------------|----------------|------------------|----------------|-----------|
| 8.0         | 187.1132       | 187.1117         | M8             | 4000/135  |
| 18.2        | 187.1151       | 187.1117         | M8             | 4000/150  |
| 5.3         | 187.1127       | 187.1117         | M8             | 4000/175  |
| 7.5         | 187.1131       | 187.1117         | M8             | 4000/250  |
| 5.3         | 169.1021       | 169.1012         | M8 fragments   | 4000/135  |
| 14.2        | 169.1036       | 169.1012         | M8 fragments   | 4000/150  |
| 0.0         | 169.1012       | 169.1012         | M8 fragments   | 4000/175  |
| 18.9        | 169.1044       | 169.1012         | M8 fragments   | 4000/250  |
| 6.4         | 141.0708       | 141.0699         | M8 fragments   | 4000/135  |
| 15.6        | 141.0721       | 141.0699         | M8 fragments   | 4000/150  |
| -5.0        | 141.0692       | 141.0699         | M8 fragments   | 4000/175  |
| 24.1        | 141.0733       | 141.0699         | M8 fragments   | 4000/250  |
| 34.3        | 105.0371       | 105.0335         | M8 fragments   | 4000/250  |
| 24.8        | 105.0361       | 105.0335         | M8 fragments   | 4000/150  |
| 24.8        | 105.0361       | 105.0335         | M8 fragments   | 4000/175  |
| 52.4        | 105.039        | 105.0335         | M8 fragments   | 4000/250  |
| 8.8         | 91.055         | 91.0542          | tropylium      | 4000/135  |
| 19.8        | 91.056         | 91.0542          | tropylium      | 4000/150  |
| -2.2        | 91.054         | 91.0542          | tropylium      | 4000/175  |
| 26.4        | 91.0566        | 91.0542          | tropylium      | 4000/250  |
| -107.7      | 205.124        | 205.1461         | M8 NH4 adduct  | 4000/135  |
| -99.0       | 205.1258       | 205.1461         | M8 NH4 adduct  | 4000/150  |
| -113.1      | 205.1229       | 205.1461         | M8 NH4 adduct  | 4000/175  |
| 8.3         | 205.124        | 205.1223         | M8 -H2O adduct | 4000/135  |
| 17.1        | 205.1258       | 205.1223         | M8 -H2O adduct | 4000/150  |
| 2.9         | 205.1229       | 205.1223         | M8 -H2O adduct | 4000/175  |

**Supplemental Figure 8b. LC-TOF-MS of CPK metabolite M7 showing effect of fragmentation voltage variation on ion at  $m/z$  205 and weaker formation of ions at  $m/z$  169, 141 and 91**

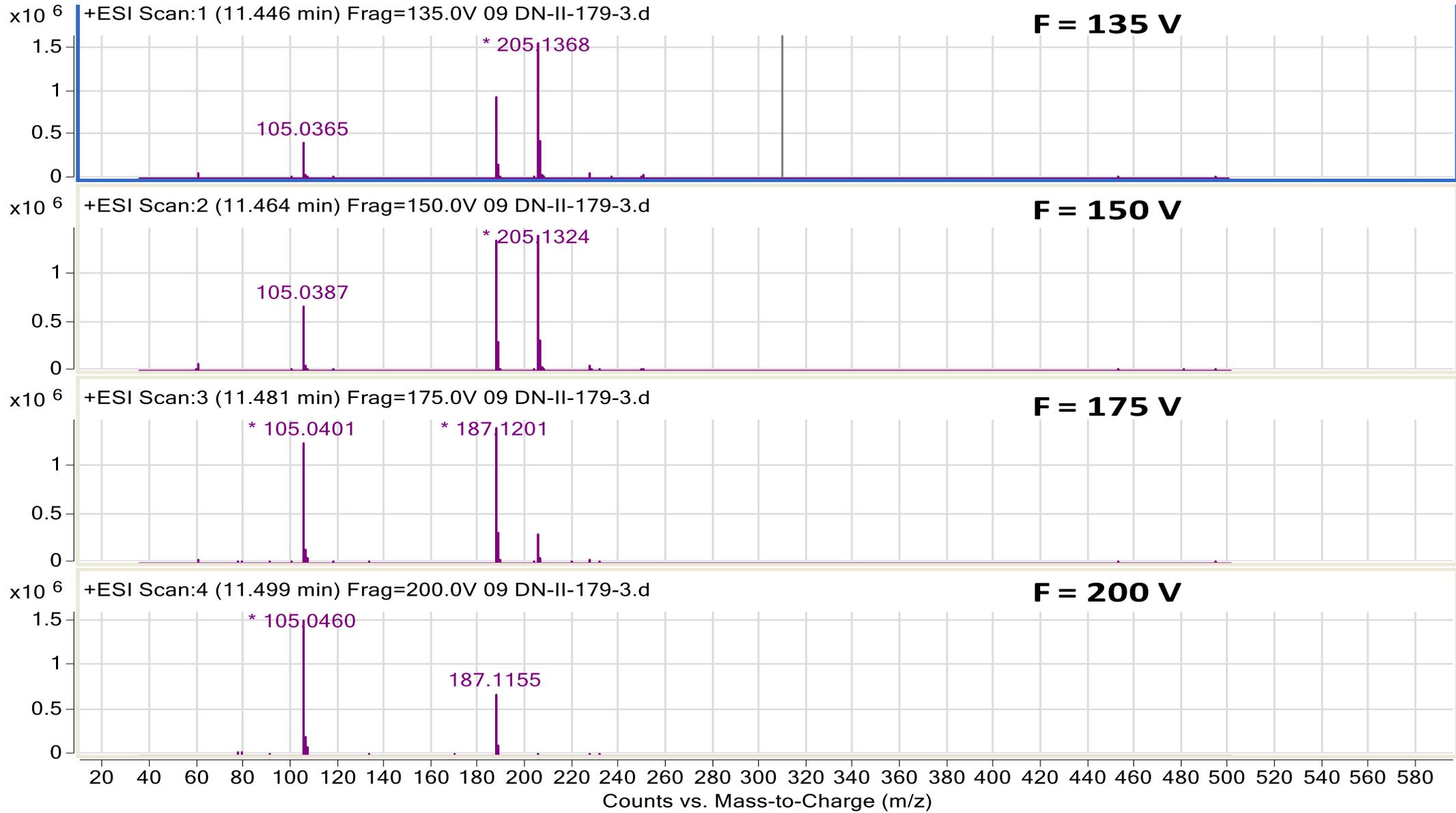
# M7



| Error (ppm) | Measured (m/z) | Calculated (m/z) | M7             | ESI/F (V) |
|-------------|----------------|------------------|----------------|-----------|
| 10.2        | 187.1136       | 187.1117         | M7             | 4000/135  |
| 16.0        | 187.1147       | 187.1117         | M7             | 4000/150  |
| 5.9         | 187.1128       | 187.1117         | M7             | 4000/175  |
| 7.5         | 187.1131       | 187.1117         | M7             | 4000/250  |
| 27.6        | 105.0364       | 105.0335         | M7 fragments   | 4000/250  |
| 40.9        | 105.0378       | 105.0335         | M7 fragments   | 4000/150  |
| 27.6        | 105.0364       | 105.0335         | M7 fragments   | 4000/175  |
| 45.7        | 105.0383       | 105.0335         | M7 fragments   | 4000/250  |
| -103.8      | 205.1248       | 205.1461         | M7 NH4 adduct  | 4000/135  |
| -101.4      | 205.1253       | 205.1461         | M7 NH4 adduct  | 4000/150  |
| -112.6      | 205.123        | 205.1461         | M7 NH4 adduct  | 4000/175  |
| 12.2        | 205.1248       | 205.1223         | M7-H2O adduct  | 4000/135  |
| 14.6        | 205.1253       | 205.1223         | M7 -H2O adduct | 4000/150  |
| 3.4         | 205.123        | 205.1223         | M7-H2O adduct  | 4000/175  |

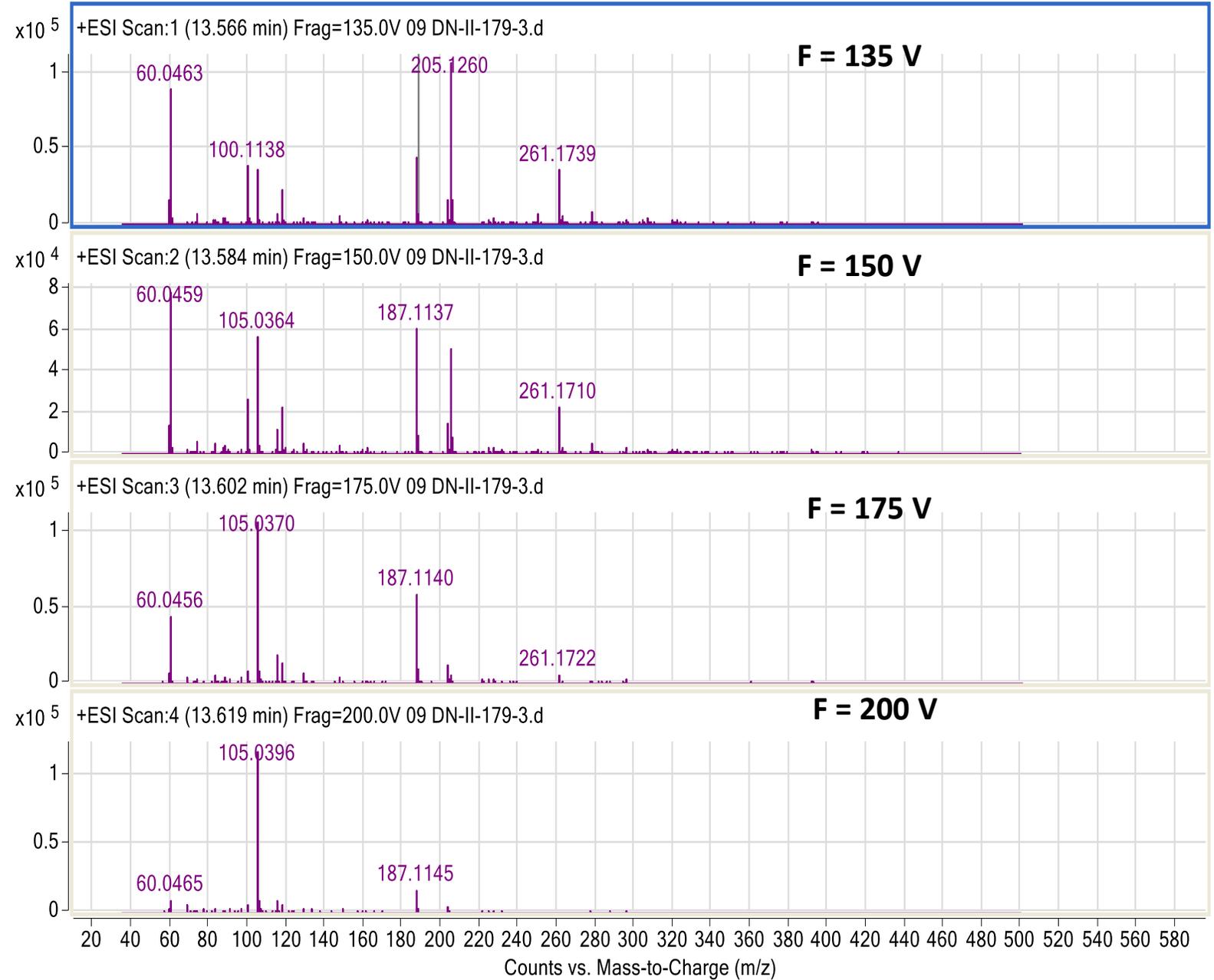
**Supplemental Figures 9a and 9b. LC-TOF-MS of CPK metabolites M6 and M9 showing effect of fragmentation voltage variation on ion at  $m/z$  205**

# M6



# M9

| Error(ppm) | Measured (m/z) | Calculated (m/z) |                | ESI/F (V) |
|------------|----------------|------------------|----------------|-----------|
| 8.0        | 187.1132       | 187.1117         | M9             | 4000/135  |
| 18.2       | 187.1151       | 187.1117         | M9             | 4000/150  |
| 5.3        | 187.1127       | 187.1117         | M9             | 4000/175  |
| 8.6        | 187.1133       | 187.1117         | M9             | 4000/250  |
| 36.2       | 105.0373       | 105.0335         | M9 fragments   | 4000/250  |
| 37.1       | 105.0374       | 105.0335         | M9 fragments   | 4000/150  |
| 63.8       | 105.0402       | 105.0335         | M9 fragments   | 4000/175  |
| 100.0      | 105.044        | 105.0335         | M9 fragments   | 4000/250  |
| -42.4      | 205.1374       | 205.1461         | M9 NH4 adduct  | 4000/135  |
| -80.4      | 205.1296       | 205.1461         | M9 NH4 adduct  | 4000/150  |
| -102.9     | 205.125        | 205.1461         | M9 NH4 adduct  | 4000/175  |
| 73.6       | 205.1374       | 205.1223         | M9 -H2O adduct | 4000/135  |
| 35.6       | 205.1296       | 205.1223         | M9 -H2O adduct | 4000/150  |
| 13.2       | 205.125        | 205.1223         | M9 -H2O adduct | 4000/175  |



**Supplemental Figure 10. LC-TOF-MS Extracted ions of  $m/z$  205 (M+H; Cluster 1)**

